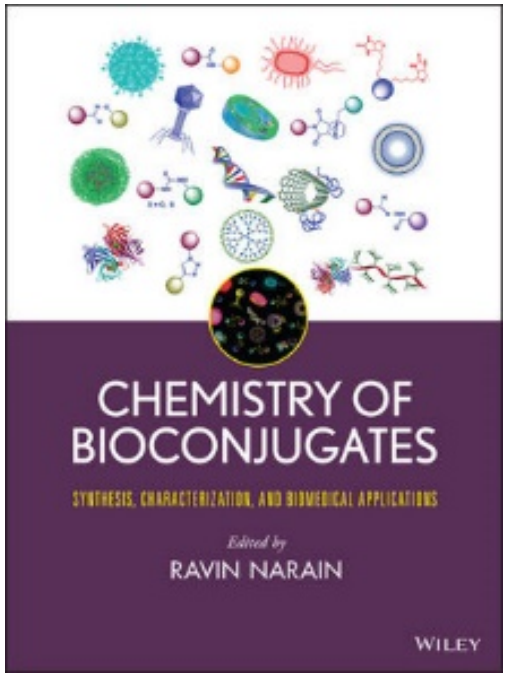
10. Konjugáty peptidů a proteinů

Konjugace je chemická strategie jak vytvořit

stabilní kovalentní či nekovalentní vazbu mezi dvěma molekulami.

Biokonjugace, je konjugace kdy alespoň jedna z molekul je biomolekula,

tzn., peptid, protein, nukleová kyselina, lipid, sacharid atd.



Kovalentní biokonjugace

(kovalentní vazba, sdílení elektronového páru, 100-400 KJ/mol)
(nekovalentní vazby do 60 KJ/mol)

Nahodilé, tj. modifikace na více místech. Často mohou vést ke ztrátě biologické aktivity biomolekuly.

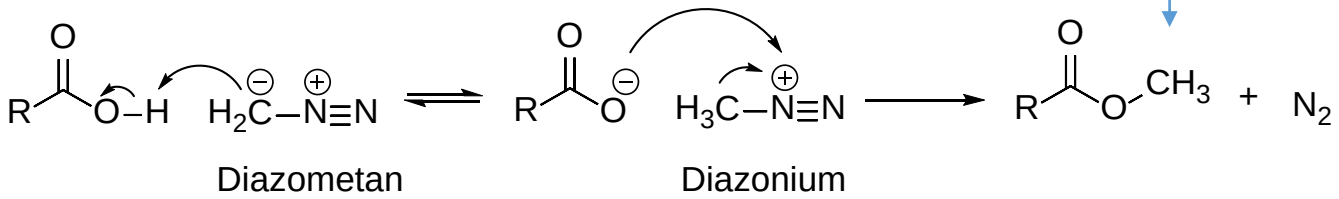
Specifické, tj. modifikace pouze jednoho místa v biomolekule.
Moderní metody tzv. *bio-ortogonálních* konjugací jsou často vysoce specifické a zachovávají bioaktivitu.

Kovalentní modifikace členíme hlavně podle toho **na jakou reaktivní chemickou skupinu je cíleno**, tzn. amin, karboxyl, aldehyd atd.

Modifikace karboxylové skupiny

Esterifikace diazoalkány

Substitucí na methyl je možno vnést další skupinu

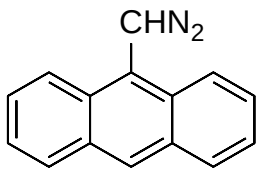


Diazometan Diazonium

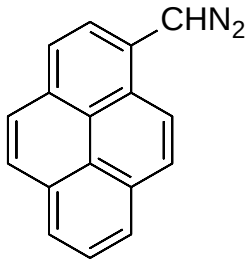
Nejprve diazomethan nukleofilně napadá karboxyl a odtrhne proton,

pak následuje nukleofilní atak karboxylátu na diazoniový kationt následovaný alkylací.

Hnací silou reakce je formování dusíku, který je skvělou odstupující skupinou.

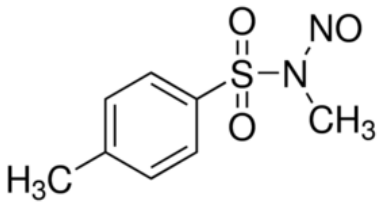


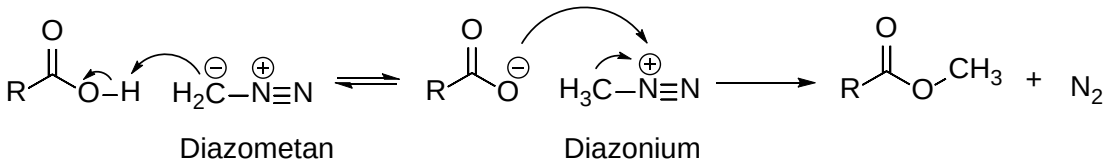
ADAM



PDAM

Fluorescenční deriváty diazomethanu
používané např. pro značení mastných kyselin.

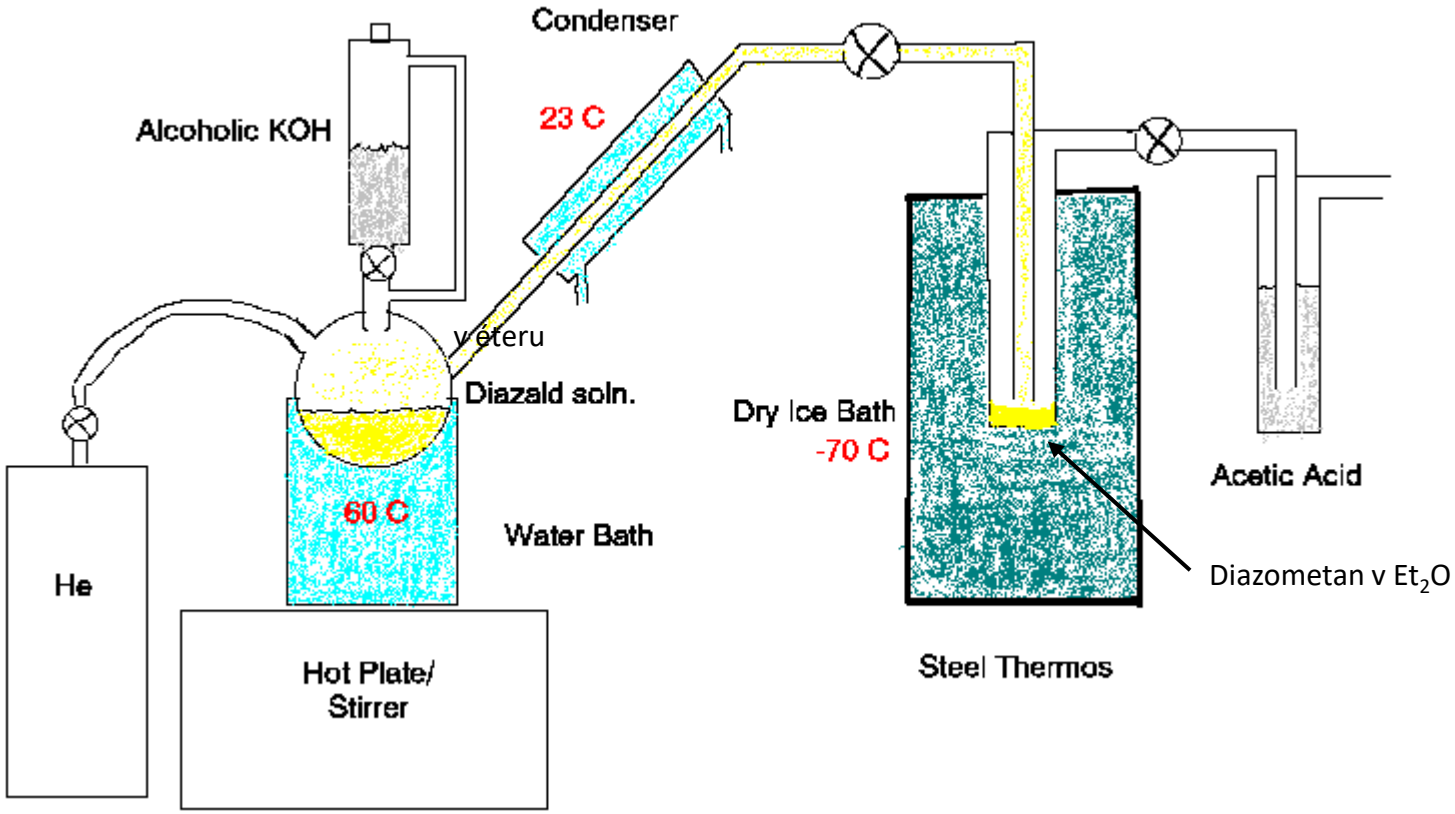




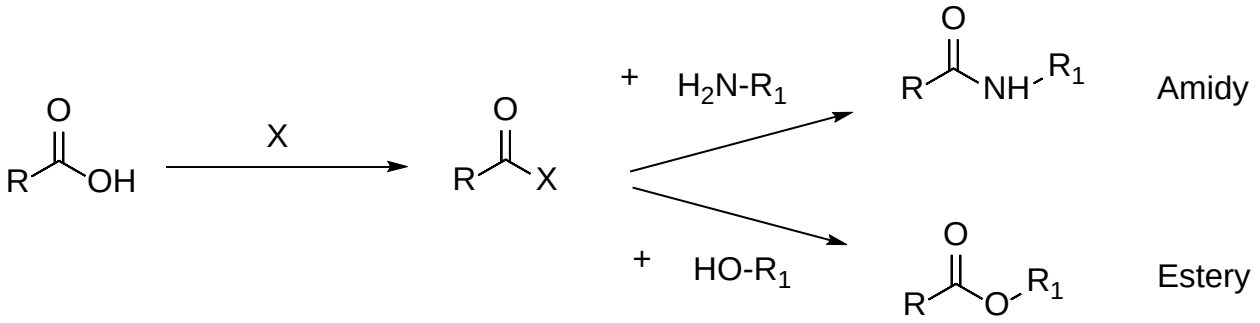
Diazald
4-methyl-N-(p-tolylsulfonyl)nitrosamide pro přípravu diazomethanu.

V chemii oblíbená reakce pro přípravu methyl esterů, diazometan je ale výbušný

Laborartorní příprava diazomethanu

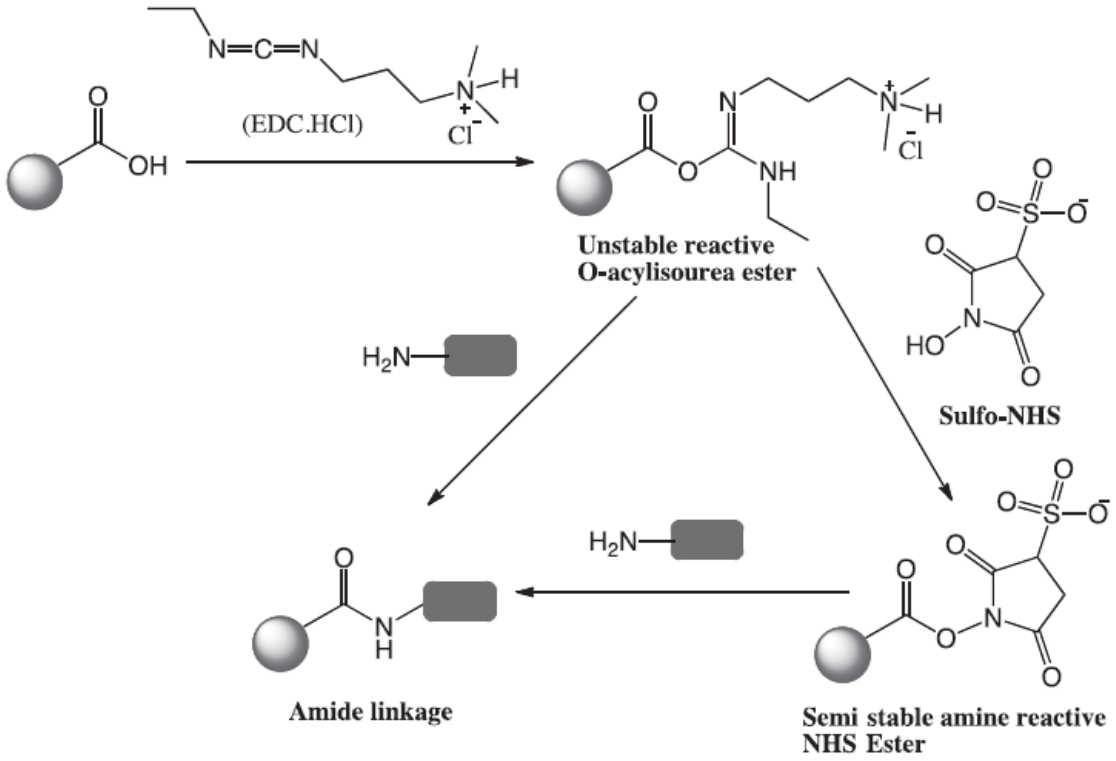


Aktivace karboxylu pro reakci s aminy a alkoholy



X je aktivační činidlo, pro biokonjugace obvykle používáme karbodiimidy

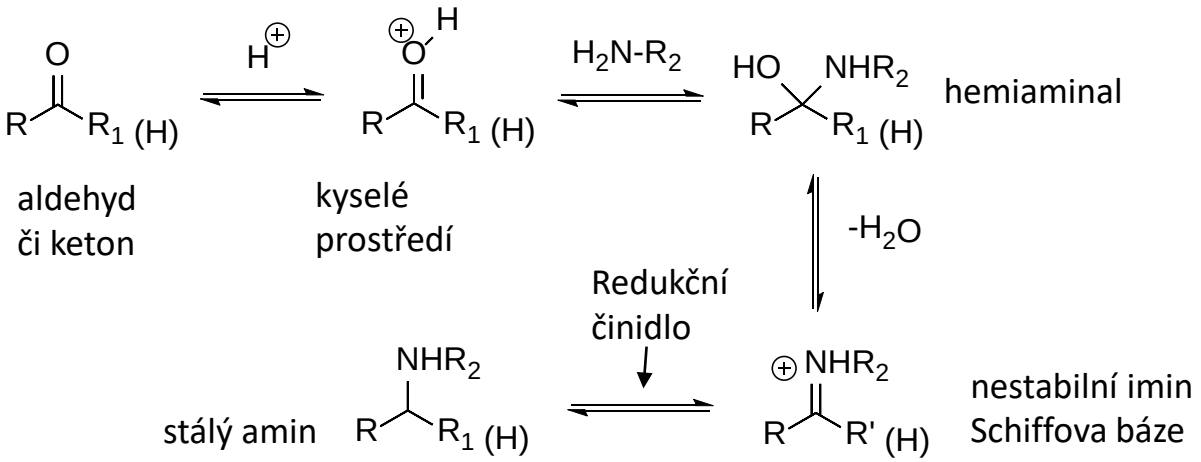
Nejčastěji používaným karbodiimidem pro biokonjugace je vodorozpustný EDC (či EDAC)



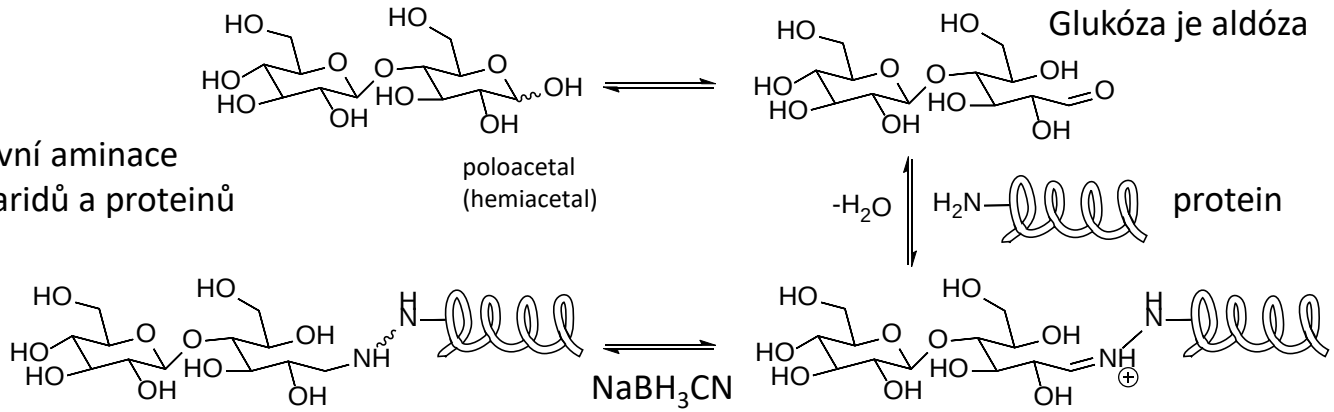
Modifikace karbonylové skupiny

Aldehydy a ketony jsou dobrými elektrofily, takže dobře podléhají nukleofilním adicím s nukleofily, jako jsou aminy, hydrazidy či hydraziny.

Konjugace
reduktivní aminací

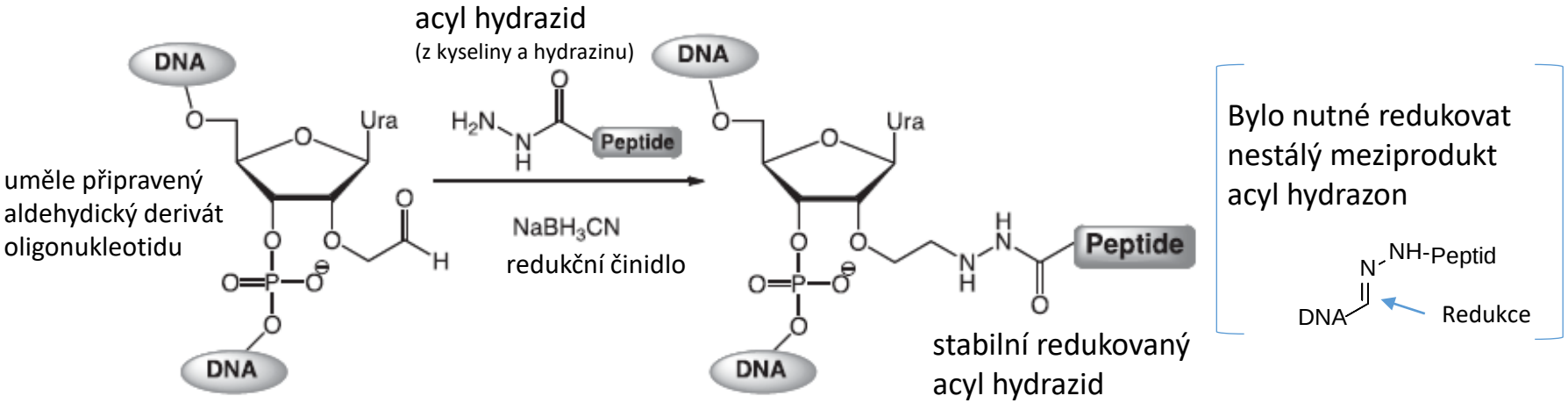


Příklad použití reduktivní aminace pro biokonjugaci sacharidů a proteinů

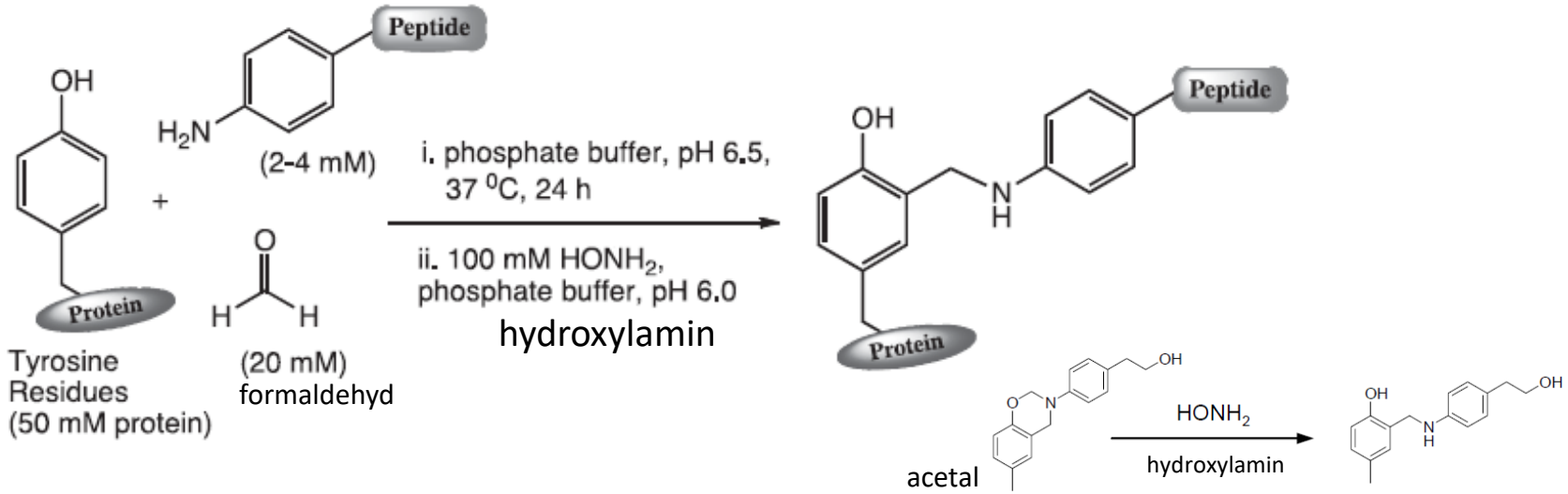


Diabetické patie

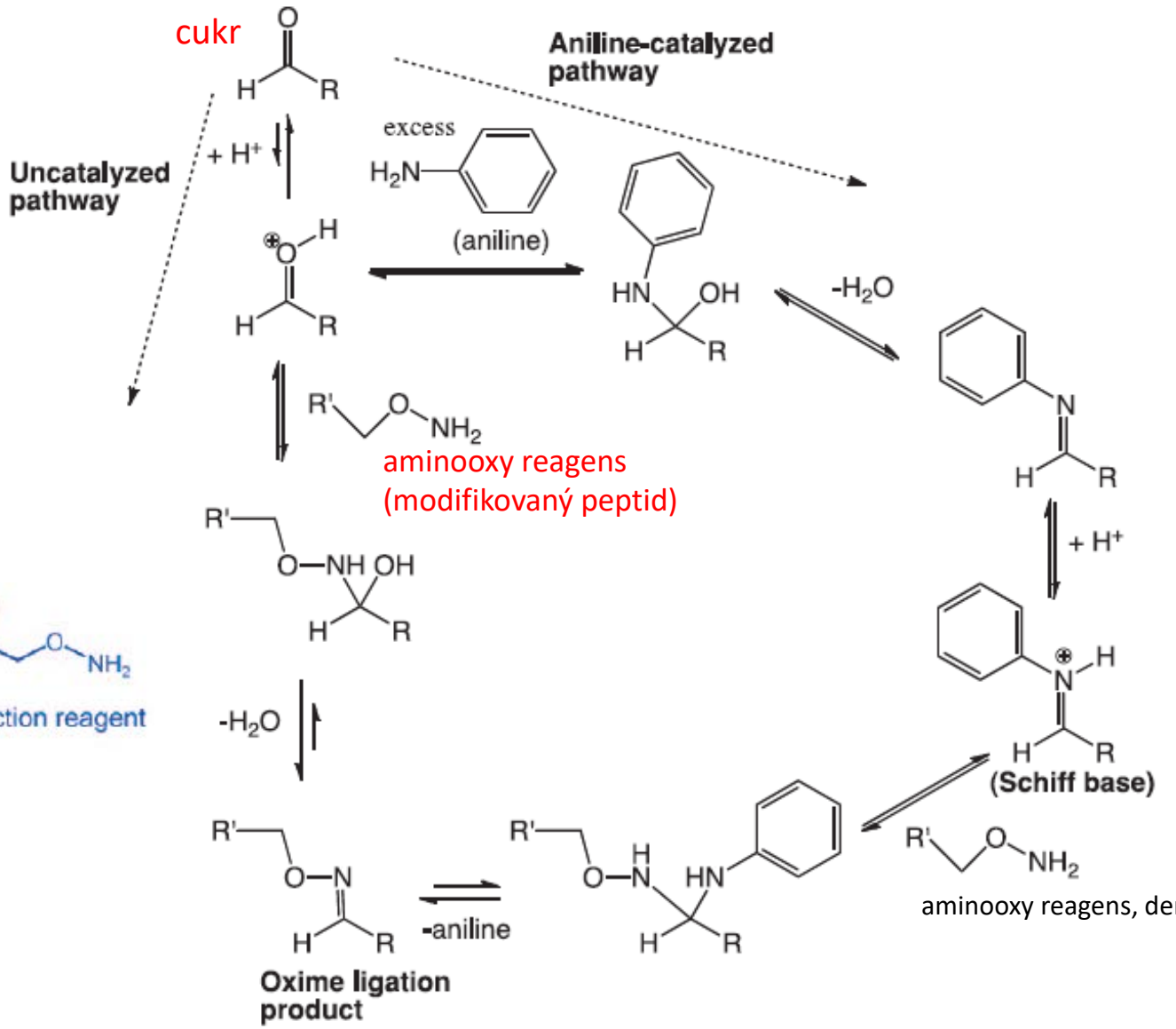
Konjugace tvorbou *hydrazonů a hydrazinů*, asi nejstarší metoda biokonjugace



Konjugace *Mannichovou reakcí*, 3-komponentní kondenzace, spojení peptidu a proteinu

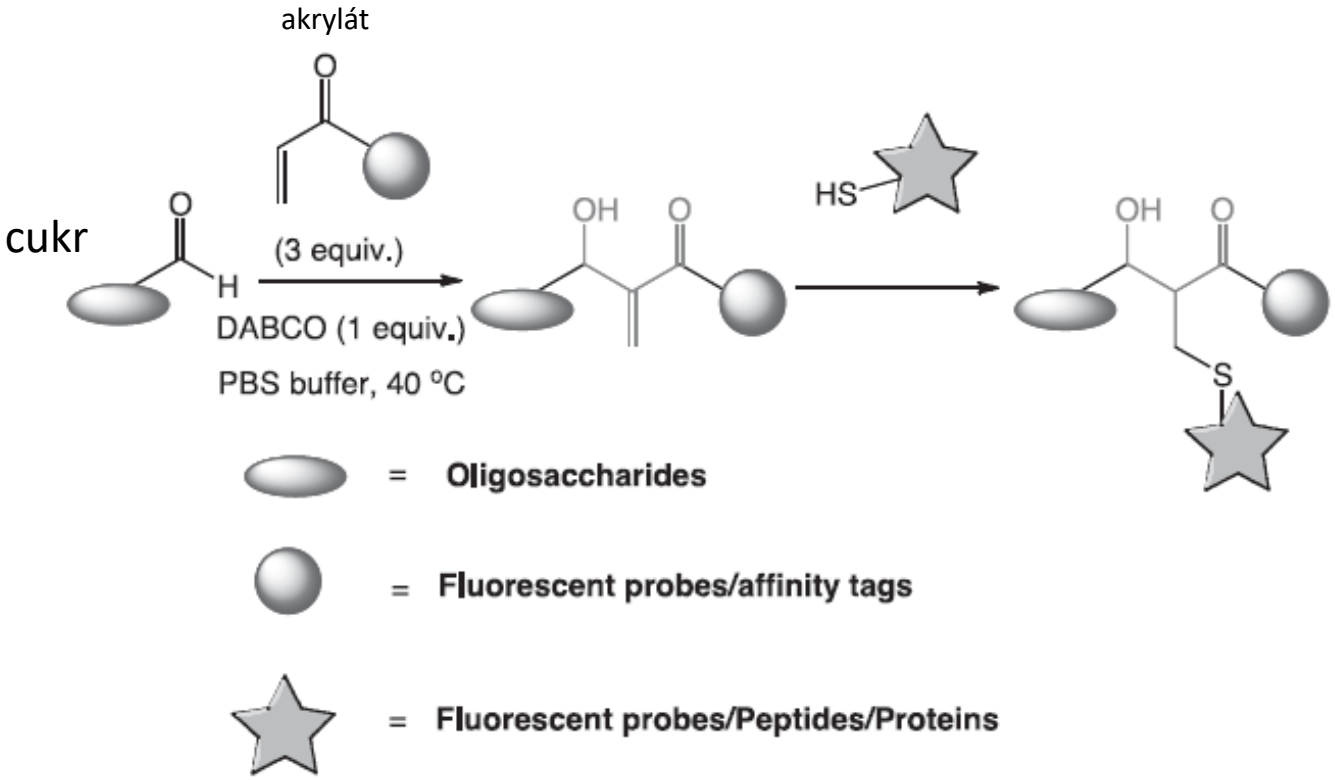


Konjugace tvorbou *oximů*, používaná pro glykosylace peptidů, či jiných látek

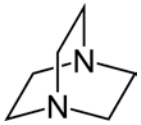


peptid

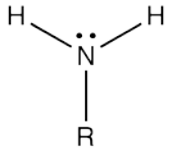
Konjugace *Morita-Baylis-Hillman (MBH) reakcí*, probíhá ve vodě,
vhodná pro tvorbu multifunkčních konjugátů



katalýza aminem DABCO

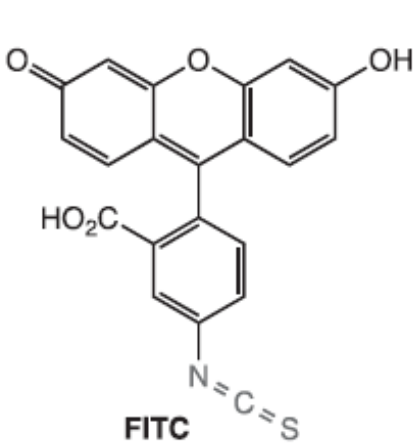


Modifikace aminoskupiny

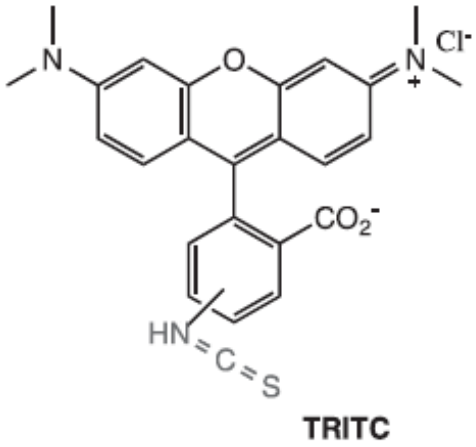


Díky volnému elektronovému páru na dusíku jsou aminy (Lys, N-konec), a zvláště ty primární, výbornými nukleofily a dobře reagují s elektrofily v alkylačních a acylačních reakcích, např. s halidy (R-X), isokyanáty (-N=C=O) a isothiokyanáty (-N=C=S).

např. fluorescenční činidla

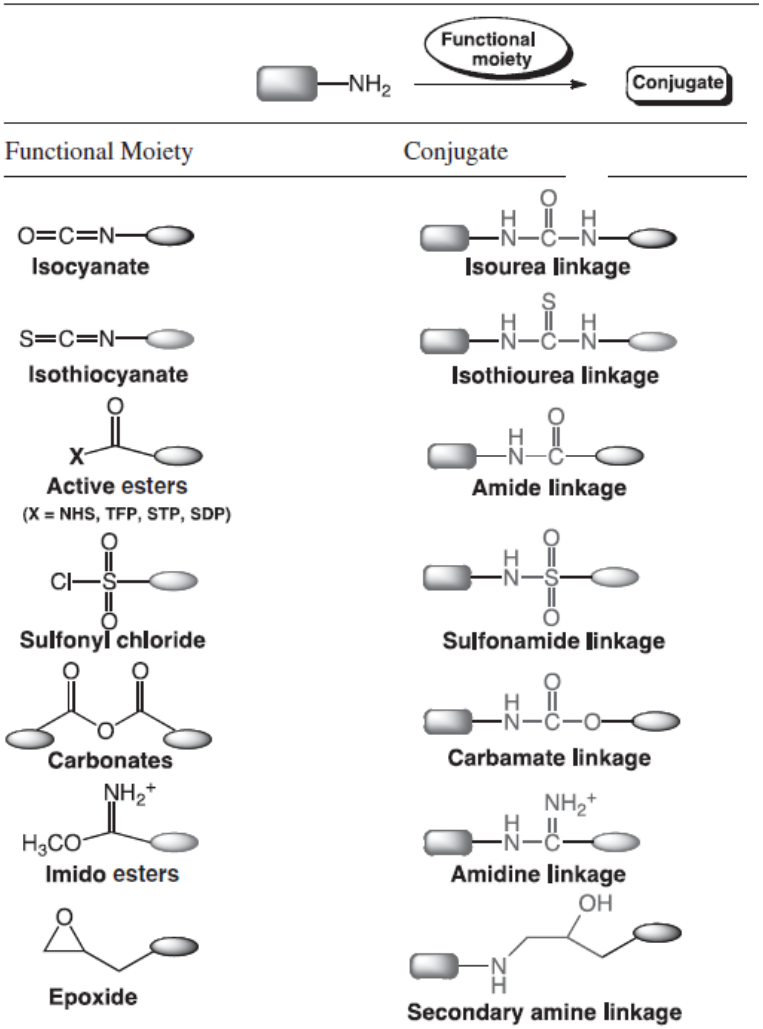


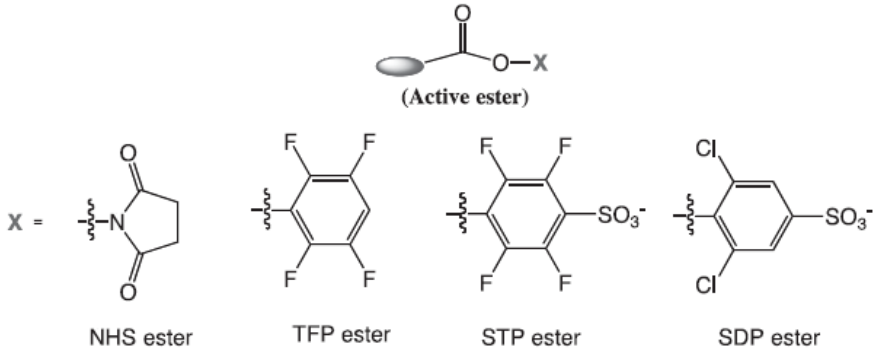
FITC



TRITC

Konvenční činidla pro kovalentní modifikaci aminů

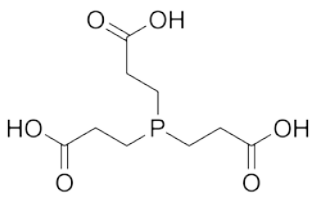




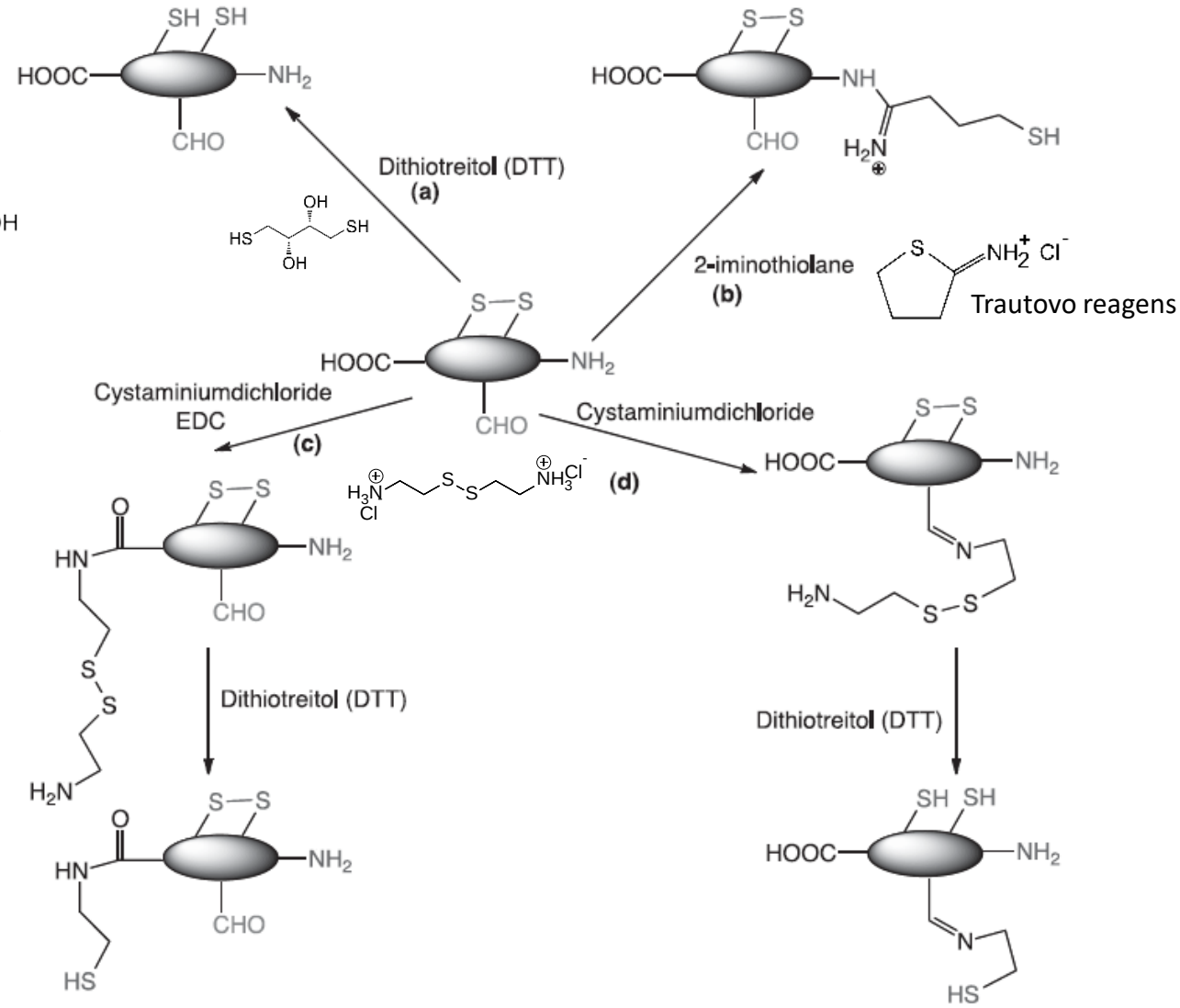
Příklady používaných aktivních esterů

Modifikace thiolové skupiny

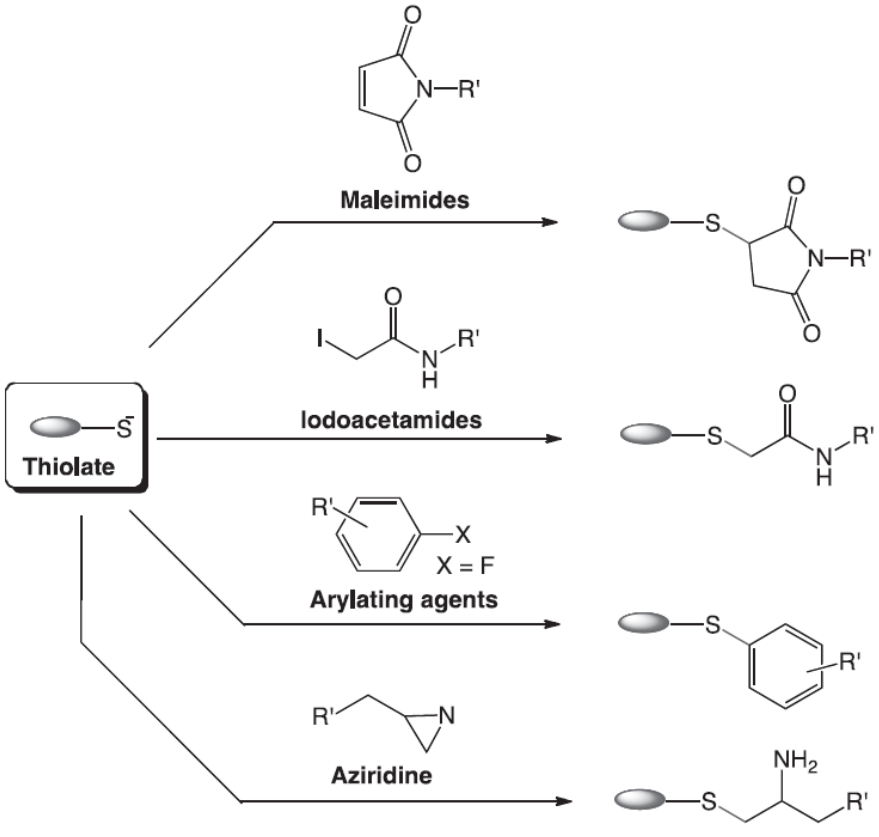
V proteinech jsou málokdy přítomny volné SH skupiny, ale mohou být inkorporovány uměle.



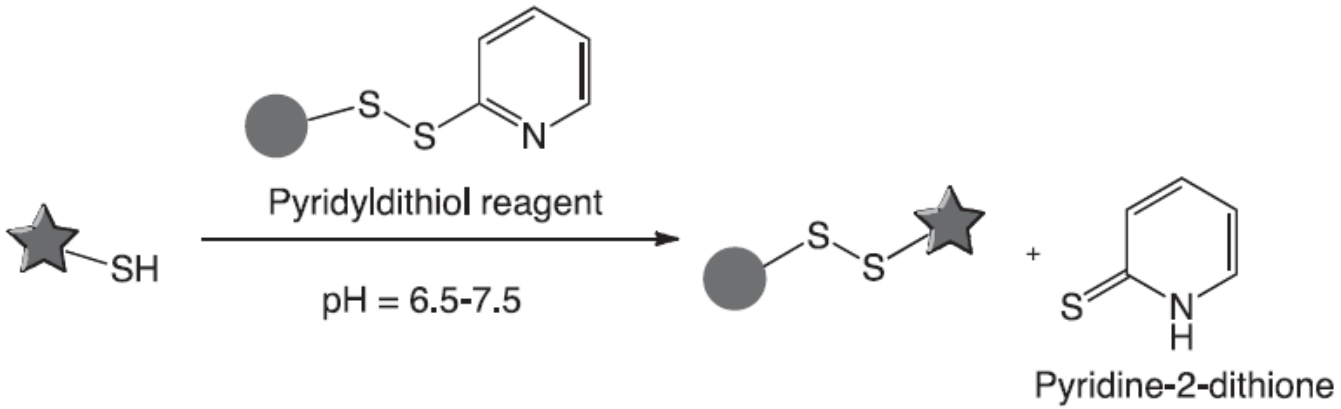
TCEP, je lepší redukční činidlo než DTT, nezapáchá, je stálý, neinterferuje s konjugací



Thioly jsou nukleofilní a výborně reagují s elektrofily



Výměna disulfidů



A_{max}=343 nm
Jeho vznik může sloužit k monitorování konjugace

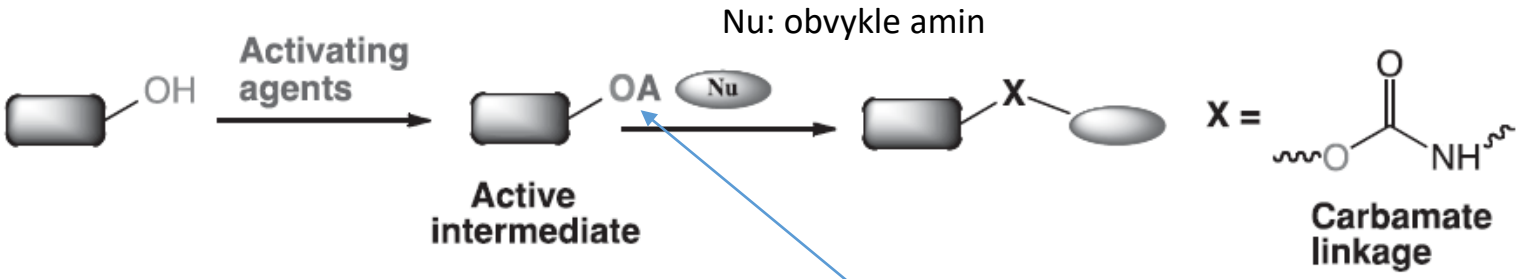
Modifikace hydroxylové skupiny

R-OH skupina je v biologických materiálech velmi častá (Ser, Thr, Tyr, cukry, nukleotidy atd.).

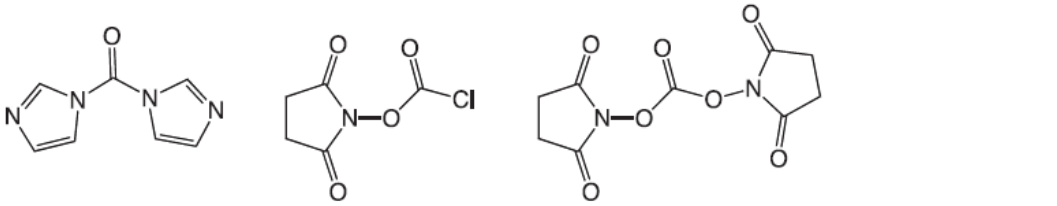
Je relativně málo nukleofilní, což činí její modifikace obtížnými, zvláště ve vodě.

Je třeba hydroxyl aktivovat.

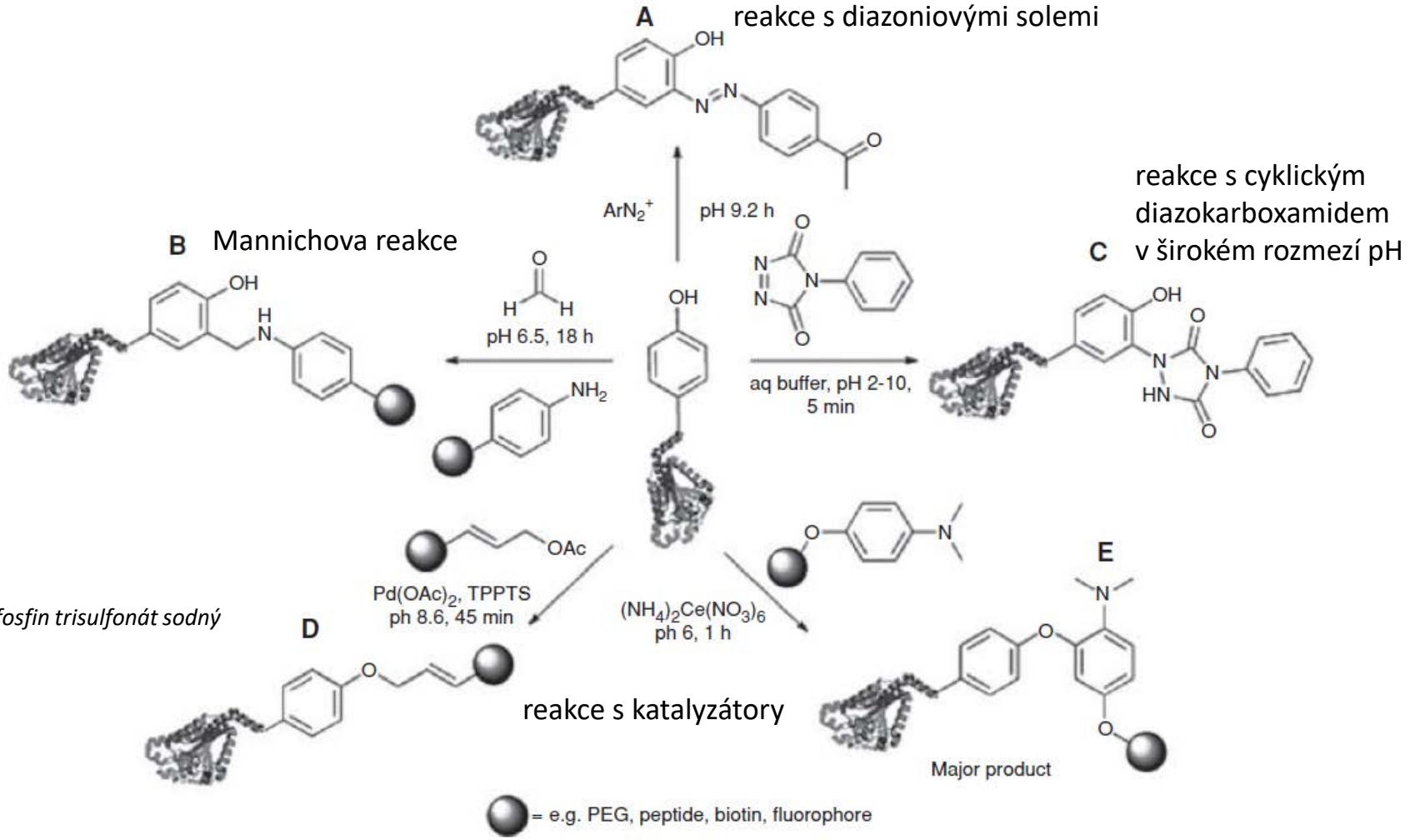
Konjugace *aktivací hydroxylu na karbamát pomocí vhodného aktivátoru*



Aktivující látky (A)



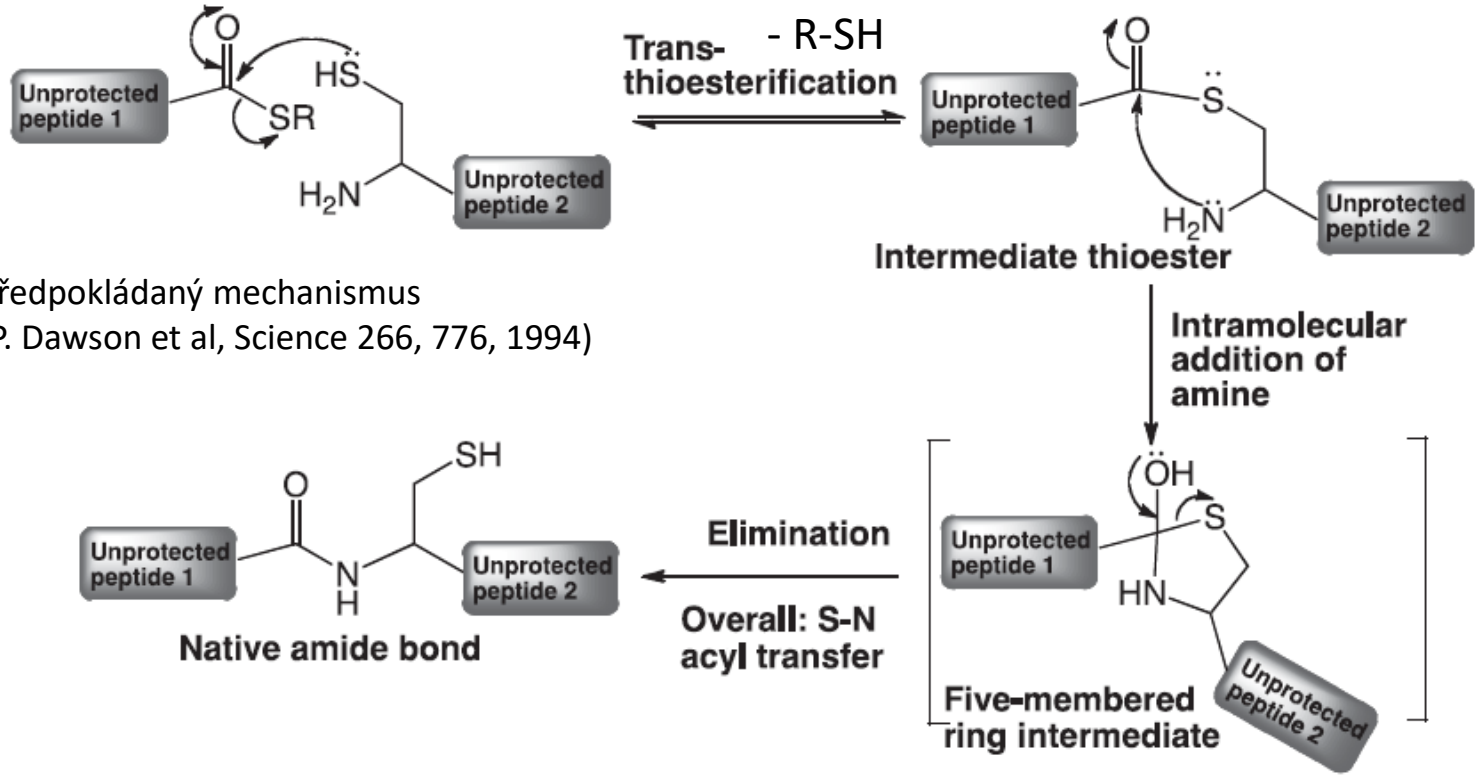
Modifikace *fenolického hydroxylu*, obvykle Tyr, bud´ na OH nebo do polohy ortho



Nativní chemické ligace

1953 Theodor Wieland: Val-CO-S-R a Cys reagovaly na Val-Cys (reakce aktivního esteru s aminem)

1990s Stephen Kent ve The Scripps Research Institute vyvinul "Native Chemical Ligation (NCL)“
na stejném principu a aplikoval jí na ligace nechráněných peptidových fragmentů a syntézu proteinů



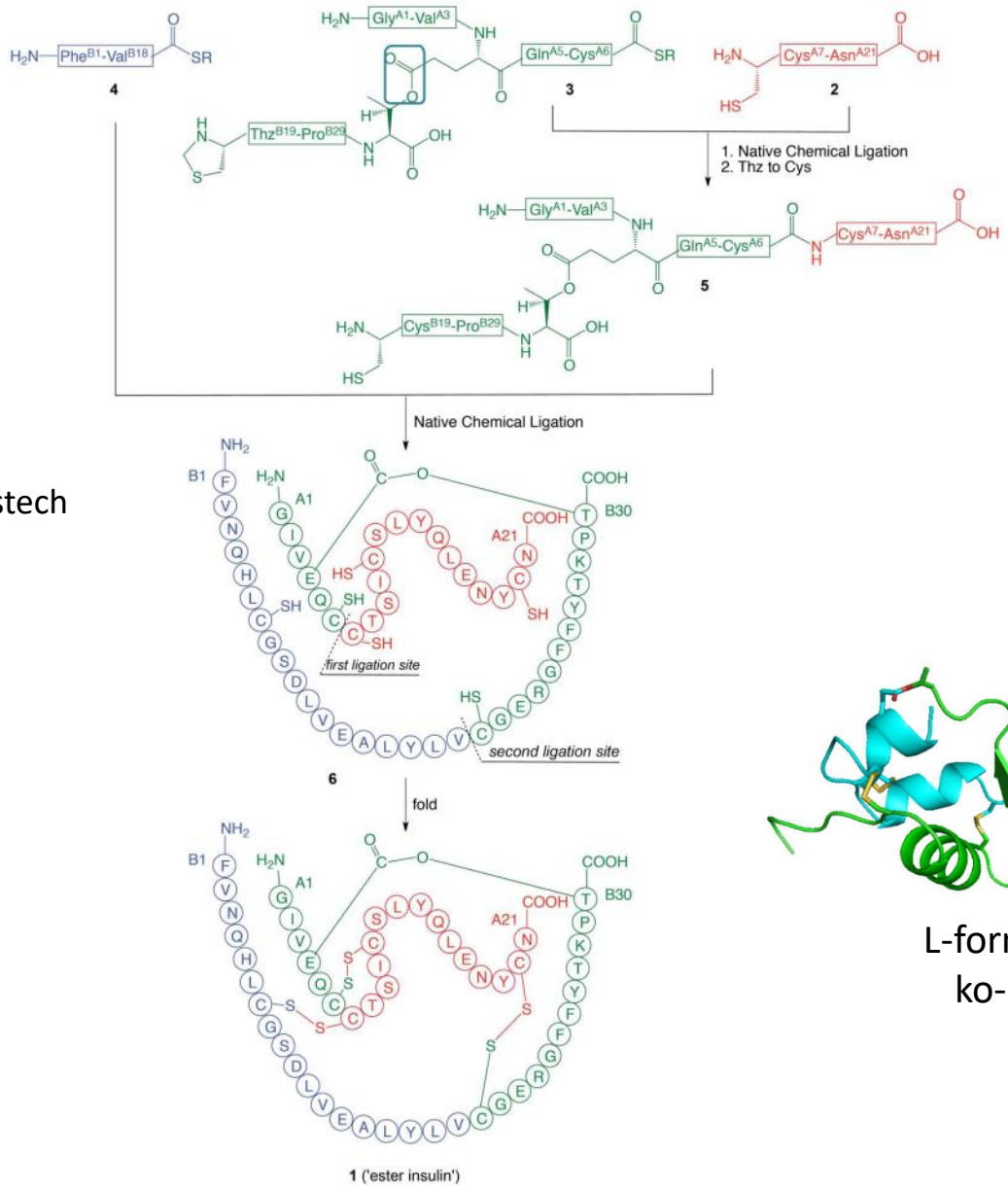
Předpokládaný mechanismus
(P. Dawson et al, Science 266, 776, 1994)

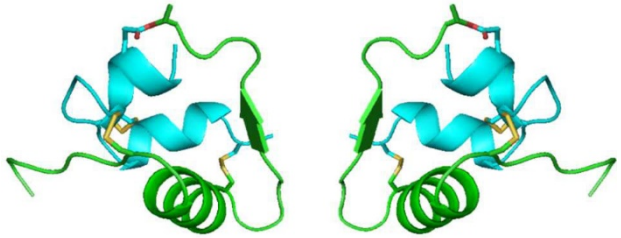
V jádru se jedná o chemoselektivní a regioselektivní transthioesterifikaci a poté S-N acyltransfer
které probíhájí ve vodě. Reakce je ale dost pomalá a má i vedlejší reakce.

Nativní chemické ligace jsou obecně limitovány chemickou syntézou C-koncových thioesterů.

S. Kent at al., Fully Convergent Chemical Synthesis of Ester Insulin: Determination of the High Resolution X-ray Structure by Racemic Protein Crystallography. J. Am. Chem. Soc. 2013, 135, 8, 3173–3185

Nativní ligace na dvou místech plus cyklizace přes ester, která usnadnila folding

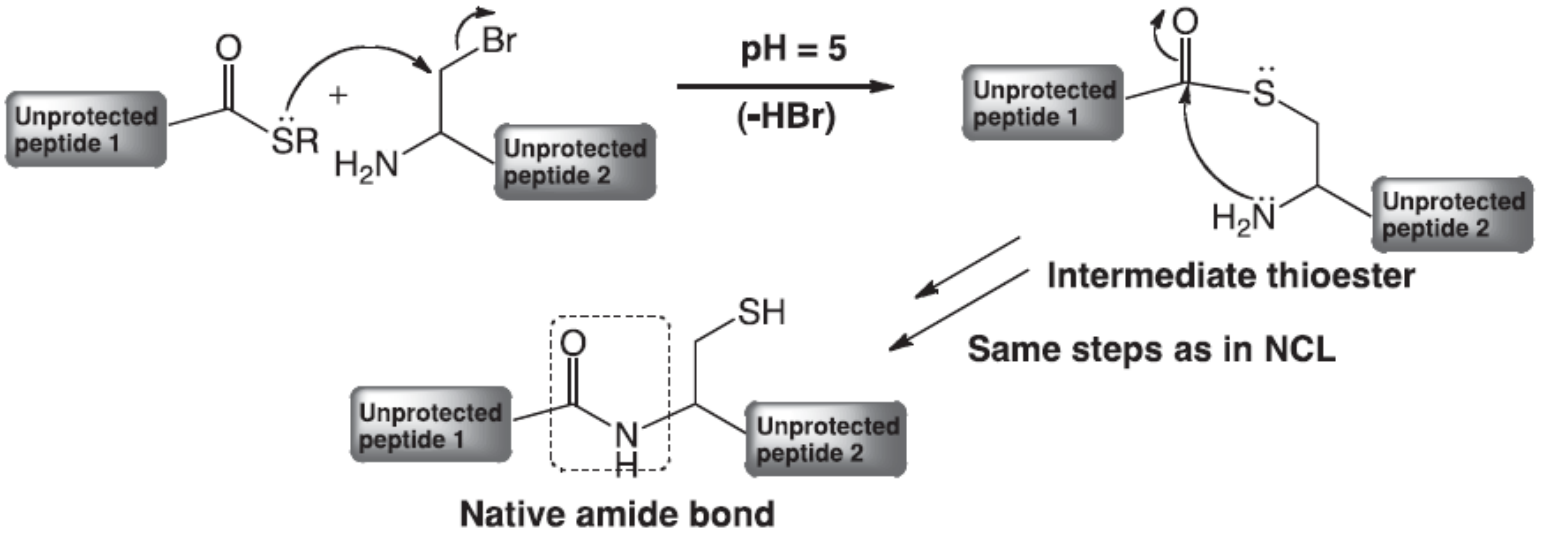




L-forma a D-forma ko-krystalizace

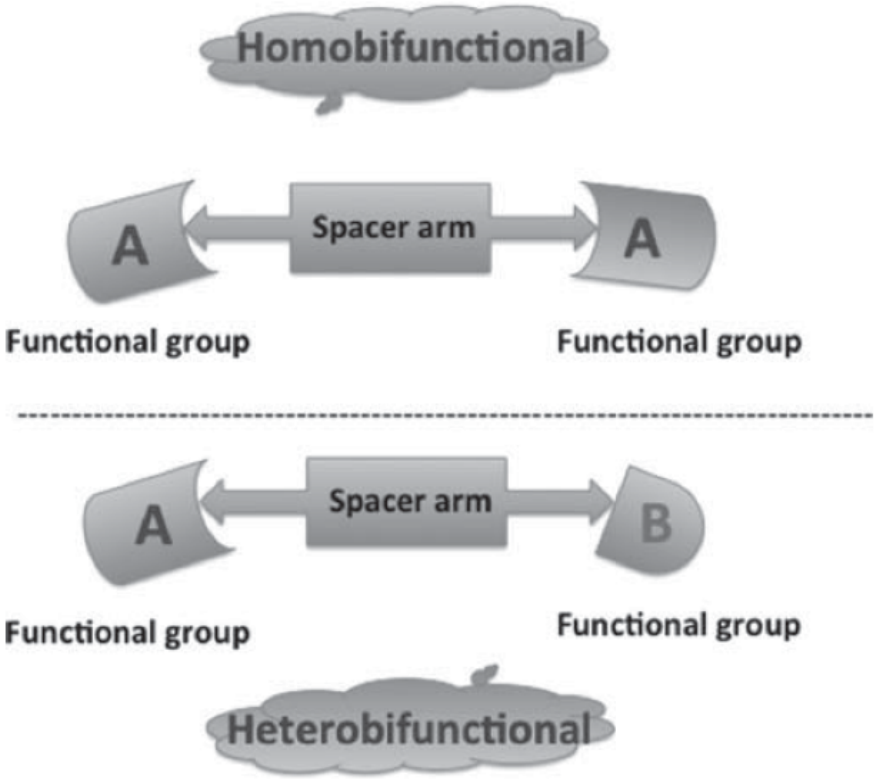
Varianta nativní chemické ligace

Tam et al., PNAS 92, 12485, 1995



„Crosslinking“ činidla pro biokonjugace

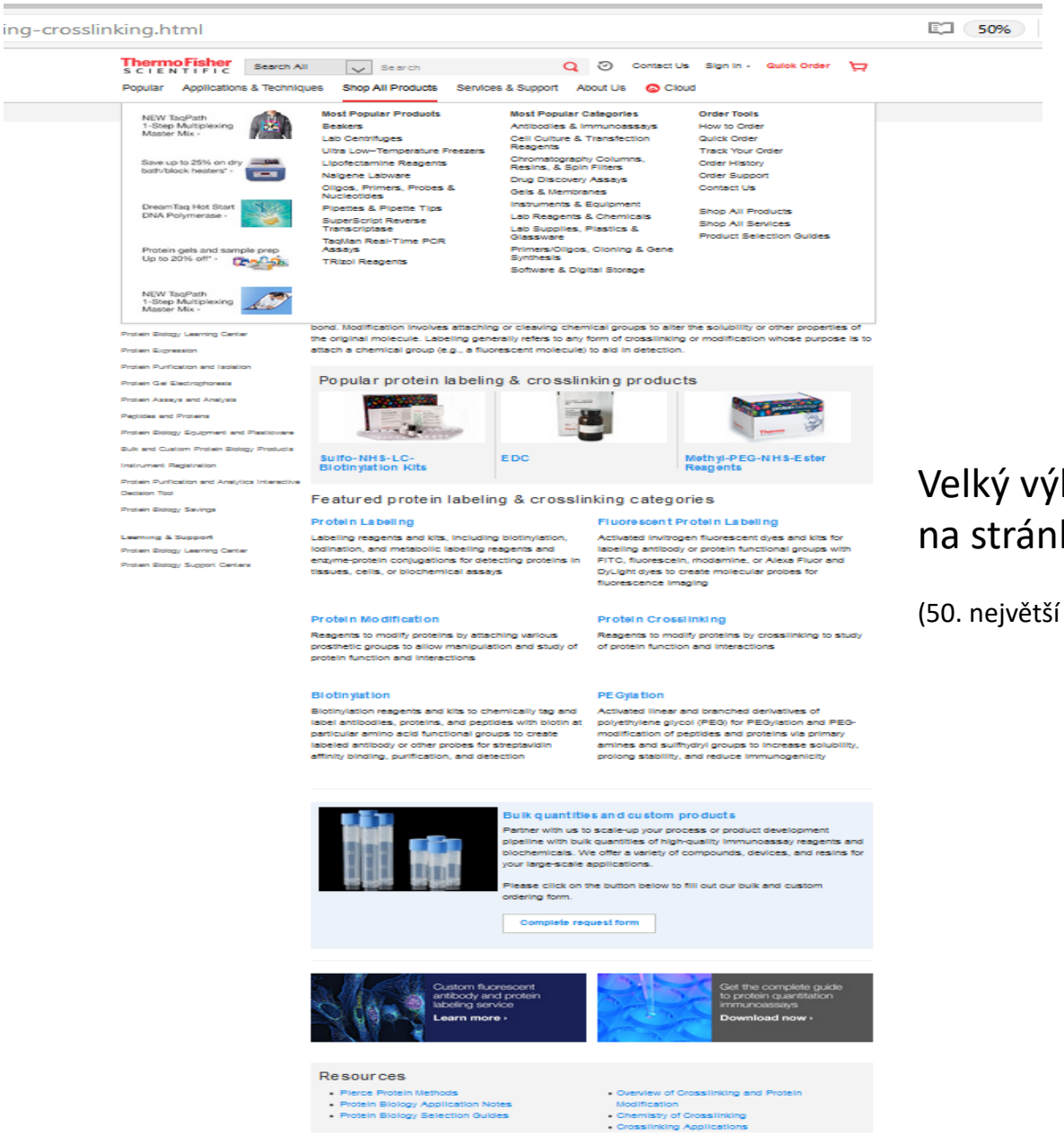
Jedná se o kovalentní spojení dvou či více molekul pomocí nějakého specifického činidla, která mohou být homobifunkční či heretobifunkční podle toho na jaké reaktivní skupiny cílí.



dvě stejné skupiny

dvě různé skupiny

https://www.thermofisher.com/cz/en/home/life-science/protein-biology/protein-labeling-crosslinking.html



Velký výběr činidel
na stránkách firmy ThermoFisher

(50. největší firma na světě, 2021)

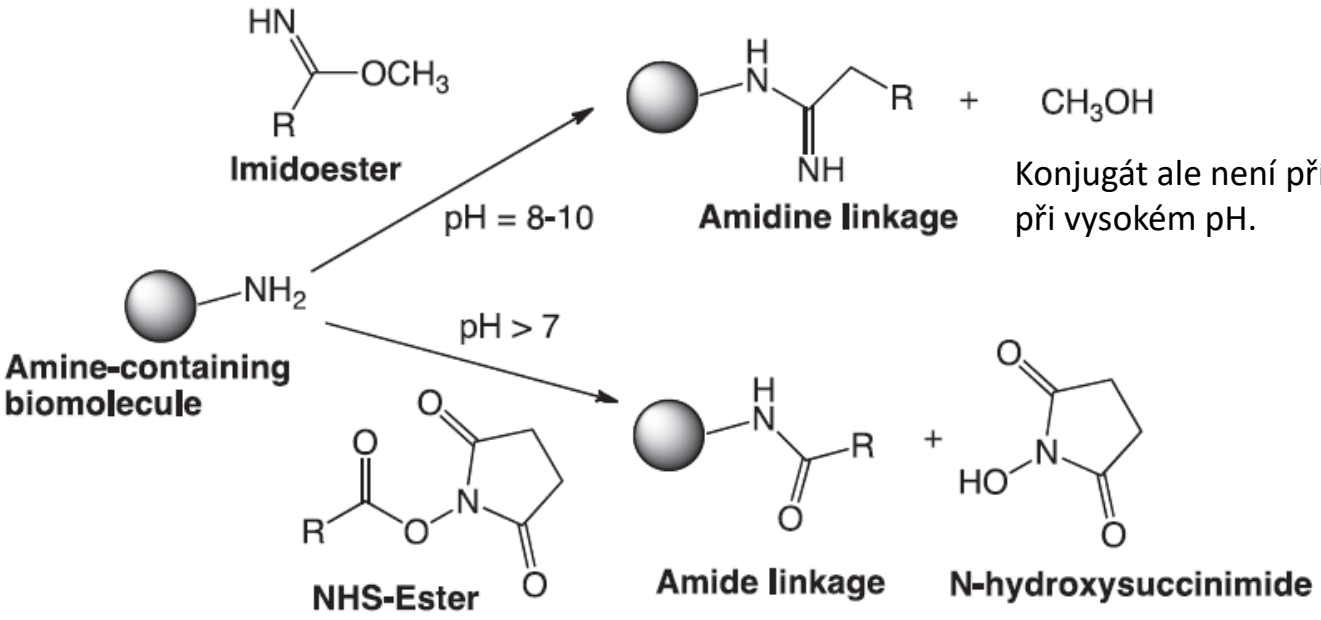
Homobifunkční činidla

Mají dvě stejné reaktivní skupiny a jsou to obvykle symetrické molekuly.
Reakce probíhá obvykle v jednom kroku.

Reakce jsou často provázeny polymerizací a self-konjugací, kterým se nedá zcela zabránit.

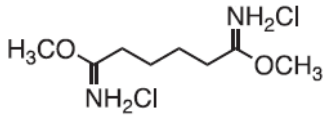
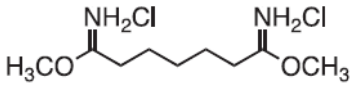
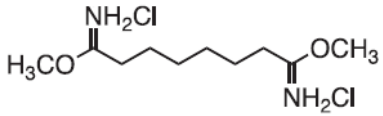
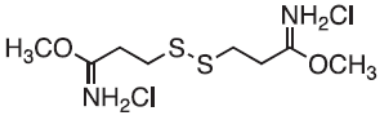
Homobifunkční činidla pro ***aminoskupiny*** jsou obvykle **imidoestery** nebo **NHS estery**.

Pozor aby v pufru nebyl Tris či glycin.



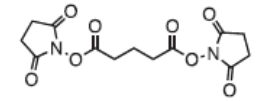
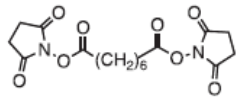
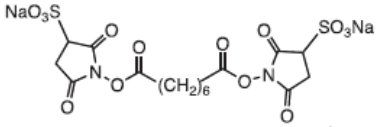
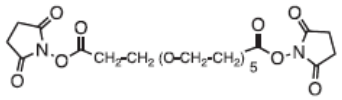
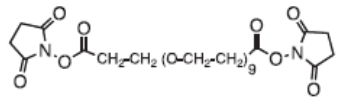
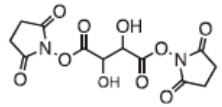
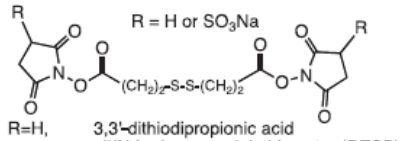
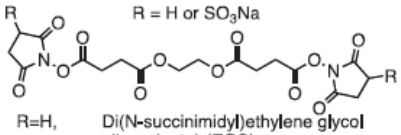
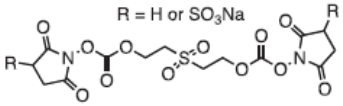
Homobifunkční činidla pro **aminy** na bázi imidoesterů.

Reagují obvykle rychle při pH 10, jsou vodorozpustné, ale nestabilní.

Chemical Structures, Names, and Abbreviations	Characteristics/Applications
 Dimethyladipimidate hydrochloride (DMA)	– Six-atom spacer arm – Noncleavable – Water soluble – Reacts rapidly at pH 8–10 – Reversible at high pH values – Retains charge character – For study of quaternary structure of proteins
 Dimethylpimelimidate hydrochloride (DMP)	– Seven-atom spacer arm – Noncleavable – Water soluble – Reacts at pH 8–10 – Reversible at high pH values – Retains charge character
 Dimethylsuberimidate hydrochloride (DMS)	– Eight-atom spacer arm – Noncleavable – Water soluble – Reacts at pH 8–10 – Reversible at high pH values – Retains charge character
 Dimethyl 3,3'-dithiobispropionimidate hydrochloride (DTBP)	– Cleavable, membrane permeable – Water soluble – Reacts at pH 8–10 – Cross-linking can be reversed with reducing agents (DTT) – Net positive charge at physiological pH – Stabilize protein–protein interactions

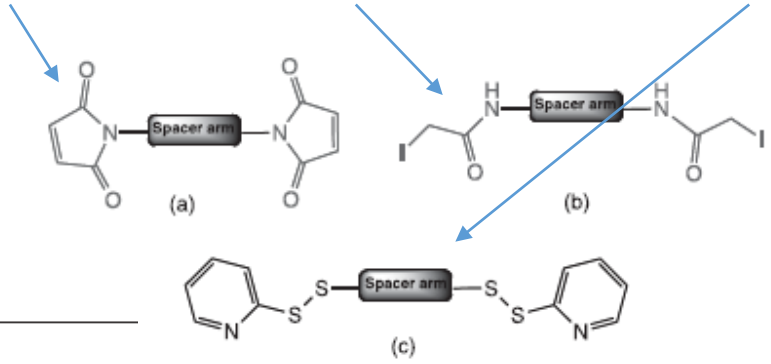
Homobifunkční činidla pro aminy na bázi NHS-esterů.

Reagují obvykle rychle při pH 10, jsou vodorozpustné, ale nestabilní.

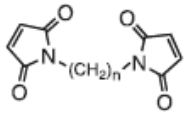
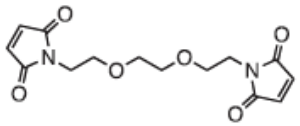
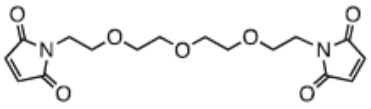
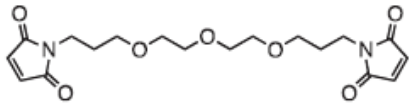
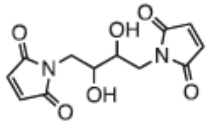
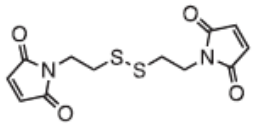
Chemical Structures, Names, and Abbreviations	Characteristics/Applications
 Disuccinimidyl glutarate (DSG)	– Noncleavable – Water insoluble – Reacts at pH 7–9 – For biomarker analysis – Capture of protein interactions on protein array surfaces
 Disuccinimidyl suberate (DSS)	– Eight-carbon spacer arm – Membrane permeable – Noncleavable – Reacts at pH 7–9 (PBS buffer) – Need to dissolve in organic solvent (DMF or DMSO)
 Bis(sulfosuccinimidyl) suberate (BS^3)	– Intracellular conjugations – Protein interaction studies – Membrane impermeable – Water soluble – Noncleavable – Proteomics applications
 Bis(succinimidyl) penta (ethyleneglycol) $BS\text{-}(PEG)_5$	– Flexible PEG spacer arm – Irreversibly cross-linking – Reacts at pH 7–9 – PEG spacer arm provides increased water solubility, stability – Reduced immunogenicity – Ideal for small molecule or peptide conjugations
 Bis(succinimidyl) nona (ethyleneglycol) $BS\text{-}(PEG)_9$	– Excellent for cross-linking cell surface proteins to stabilize protein interactions
 Disuccinimidyl tartrate (DST)	– Four-carbon spacer arm – Cleavable by periodate oxidation – Partially soluble in water – Reacts at pH 7–9 – Sulfo-DST analog also available and more water soluble – Study of protein–lipid complexes
 R=H, 3,3'-dithiodipropionic acid di(N-hydroxysuccinimide ester (DTSP) $R=SO_3Na$ 3,3'-dithio-bis-(3-sulfo-N-hydroxysuccinimidyl propionate) disodium salt (DTSSP)	– DTSP or DSP: Lomant's reagent—Eight-atom spacer arm – Membrane permeable – Reacts at pH 7–9 (PBS buffer) – Disulfide bond can be cleaved by reducing agents at pH 8.5 – Spacer arm can be cleaved with 5% beta-mercaptoethanol – DTSSP: same as above except – Membrane impermeable – Water soluble – Cell-surface cross-linking possible
 R=H, Di(N-succinimidyl)ethylene glycol disuccinate) (EGS) $R=SO_3Na$ Ehyleneglycol bis-(3-sulfo-N-hydroxysuccinimylsuccinate) disodium salt (Sulfo-EGS)	– 12-atom spacer arm – EGS is water insoluble – Cleavable by hydroxylamine at pH 8.5 for 3–6 h at 37°C – Sulfo-EGS is water soluble – Reaction conditions same as EGS – Both used for study protein interactions and large protein complexes
 R=H, Bis[2-(succinimidylcarbonyloxy) ethyl] sulphone (BSOCOES) $R=SO_3Na$ Bis[2-(sulfosuccinimidylcarbonyloxy) ethyl] sulphone (sulfo-BSOCOES)	– BSOCOES is water insoluble – Cleavable by base at pH 8.5 for 2 h at 37°C – Sulfo-BSOCOES is water soluble – Both used for studying cellular and subcellular distribution of the type II vasopressin receptor

Homobifunkční činidla pro ***thioskupiny***, obvykle maleinimidy (a), jodoacetyly (b) či pyridyldisulfidy (c).

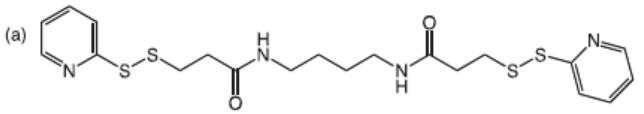
Pro konjugaci thiolů je vždy nutné odstranit všechna redukční činidla.

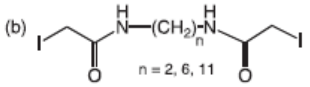


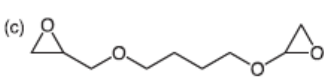
Příklady maleinimidů.

Chemical Structure, Name, and Abbreviation	Characteristics
 n = 2, 1,2-bis-maleimidoethane (BMOE) n = 4, 1,4-bis-maleimidobutane (BMB) n = 6, 1,6-bis-maleimidohexane (BMH)	– Noncleavable – Water insoluble – Reacts at pH 6.5–7.5—irreversible
 1,8-bis-maleimidotriethylene glycol (BM(PEO)$_3$)	– Polyether spacer – Water soluble – Reacts at pH 6.5–7.5 – Irreversible – Increased stability – Reduced aggregation – Less immunogenicity
 1,11-bis-maleimido-tetraethyleneglycol (BM(PEO)$_3$)	
 1,13-bis-maleimido-4,7,10-trioxadecane (BM(PEO-PPO)$_2$)	
 1,4-bis-maleimidyl-2,3-dihydroxybutane (BMDB)	– Mid-length – Reacts at pH 6.5–7.5 – Reversible cross-link – Cleavage by 15mM sodium periodate
 Dithiobismaleimidoethane (DTME)	– Intermediate length – Water insoluble – Reacts at pH 6.5–7.5 – Reversible cross-link – Cleavable by DTT or 2-mercaptoethanol

DPDPB



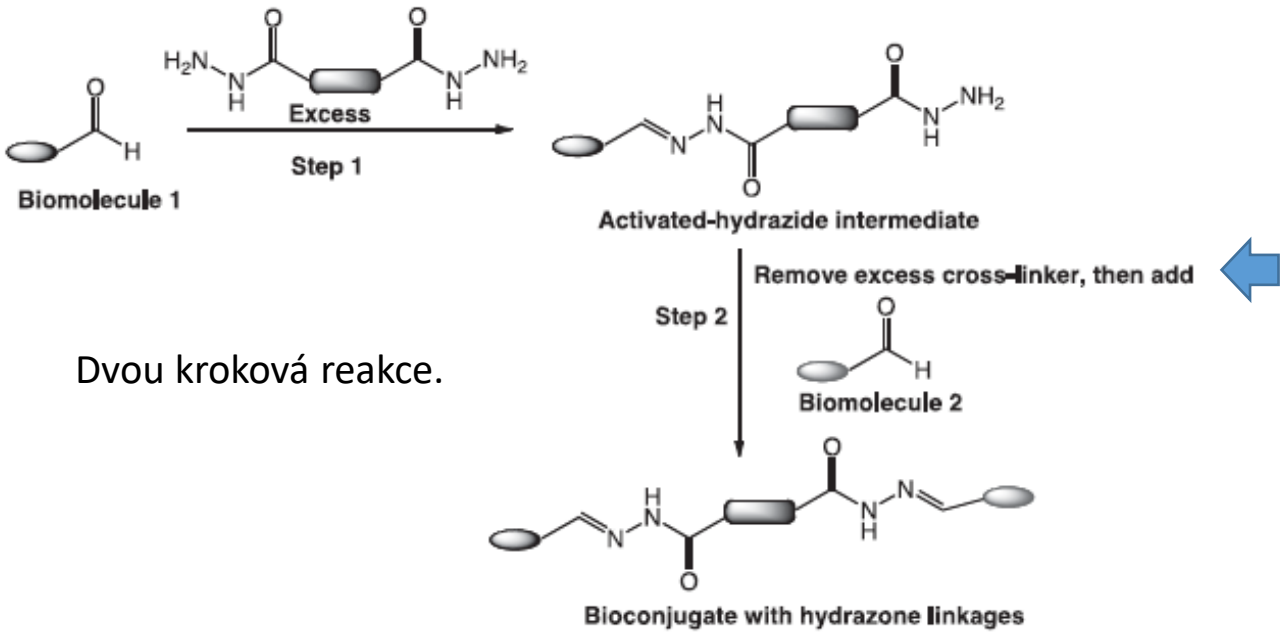




Bis-epoxidy

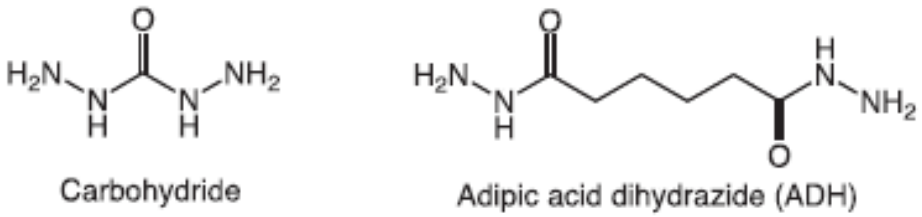
Bis-jodoacetylamid deriváty

Homobifunkční činidla pro ***aldehydy*** a ***ketony*** jsou hlavně hydrazidy.

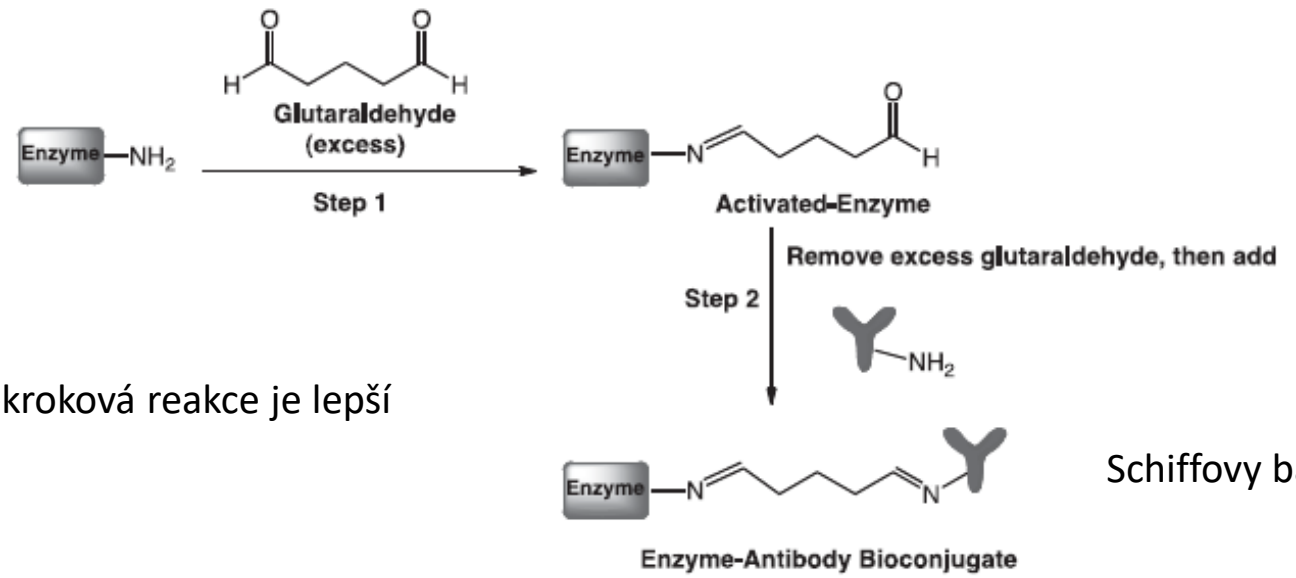


Dvou kroková reakce.

Nejčastěji se používají karbohydrid a dihydrazid adipové kyseliny (ADH).



Populární konjugace proteinů (aminů) glutaraldehydem



Dvou-kroková reakce je lepší

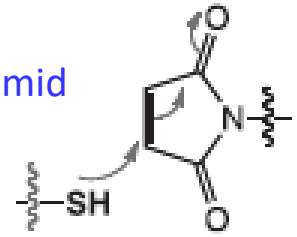
Schiffovy báze

Heterobifunkční činidla

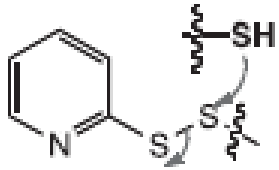
Mají dvě reaktivní skupiny. Nejprve se nechá odreagovat ta méně stálá, pak se odstraní přebytek činidla a pak se přidá druhý partner.

Výhodou je omezení polymerizací a intramolekulární modifikace.

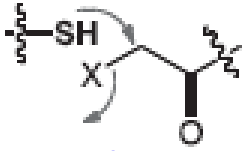
Maleinimid



A =



Pyridyldisulfid



X = Br, I

Halogen-acetyl

První reakce

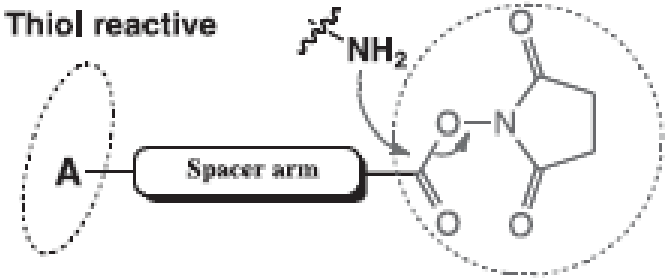
Méně stálá skupina a

Thiol reactive

Druhá reakce

Více stálá akupina a

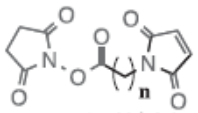
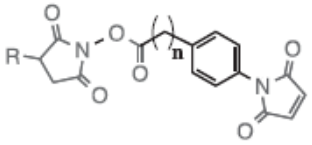
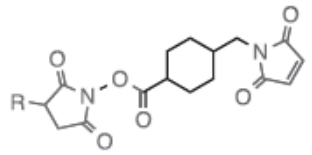
Amine reactive



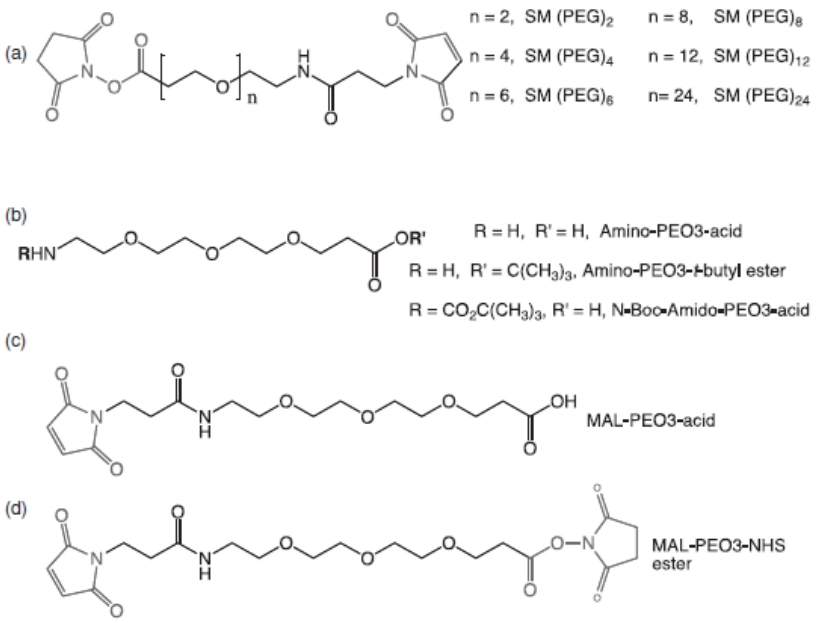
NHS-ester,

obvykle jde až do reakce s aminem.

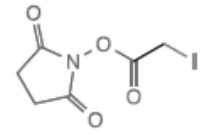
Komerčně dostupná heterobifunkční činidla na bázi maleinimidů a NHS -esterů

Chemical Structures, Names, and Abbreviations	Characteristics
 n = 1, *N*-(alpha-maleimidoacetoxy) succinimide ester (AMAS) n = 2, *N*-(beta-maleimidopropyloxy) succinimide ester (BMPS) n = 3,*N*-(gamma-maleimidobutyryloxy) succinimide ester (GMBS) n = 5, *N*-(ε-maleimidocaproyloxy) succinimide ester (EMCS)	– Noncleavable – Water insoluble – NHS ester reacts with amines at pH 7–9 – Maleimide reacts with thiols at pH 6.5–7.5 – Low potential to elicit immune response – Water-soluble Sulfo-GMBS, Sulfo-EMCS are also available. – Increased sphere of coupling for EMCS vs. GMBS
 R =H, n = 0, *m*-Maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS) R =SO_3Na, n = 0, *m*-Maleimidobenzoyl-*N*-hydroxy sulfosuccinimide ester (Sulfo-MBS) R =H, n = 3, Succinimidyl 4-(*p*-maleimidophenyl) butyrate (SMPD) R =SO_3Na, n = 3, *m*-Maleimidobenzoyl-*N*-hydroxy sulfosuccinimide ester (Sulfo-SMPD)	**MBS:** – Noncleavable – Water insoluble – For enzyme immunoconjugates **SMPD:** – Extended chain analog of MBS limits steric effect – More stable conjugates than SPDP **Sulfo-MBS and Sulfo-SMPD:** – Non cleavable – Water soluble – Membrane impermeable
 R =H, Succinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (SMCC) R =SO_3Na, Succinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (Sulfo-SMCC)	**SMCC:** – Noncleavable – Membrane permeable – NHS ester at pH 7–9 – Maleimide at pH 6.5–7.5 **Sulfo-SMCC** – Water soluble, membrane impermeable **Both SMCC and Sulfo-SMCC:** – Unusual extra stability due to cyclohexane ring – Ideal for enzyme labeling of antibodies

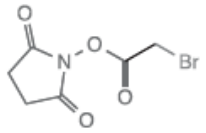
Heterobifunkční činidla s PEG



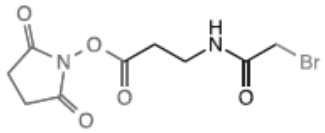
Heterobifunkční činidla na bázi NHS-esteru a jodo-či bromo-acetylu



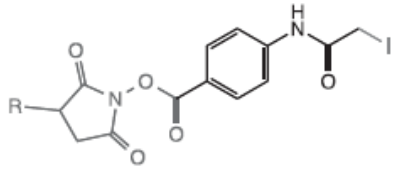
Succinimidyl iodoacetate (**SIA**)
Non cleavable and water insoluble
Preparation of protein–protein conjugates of predetermined composition (Ref. 236-237)



Succinimidyl bromoacetate (**SBA**)
Non cleavable and water insoluble
Affinity labeling of the active site of Staphylococcal nuclease (Ref. 238)



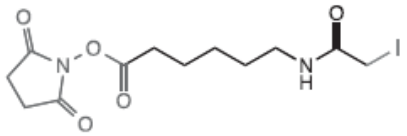
Succinimidyl-3-(bromoacetamido)propionate (**SBAP**)
Cross-link is prone to acid hydrolysis
Spacer maintains peptide-like character
Preparation of cyclic peptides and peptide conjugates (Ref. 239)



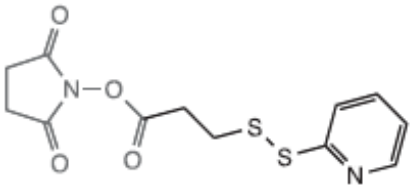
R = H, Succinimidyl (4-iodoacetyl) aminobenzoate (**SIAB**)

R = SO_3Na, Sulfosuccinimidyl (4-iodoacetyl)aminobenzoate (**Sulfo-SIAB**)

Non cleavable, water insoluble
Aminobenzoate spacer makes it membrane permeable
Sulfo-SIAB is water soluble
Used for enzyme labeling of antibodies (Ref. 240-241)



Succinimidyl-6-[(iodoacetyl)-amino]hexanoate (**SIAX**)
Water insoluble, membrane permeable
Reactivity similar to SIAB (Ref. 230)



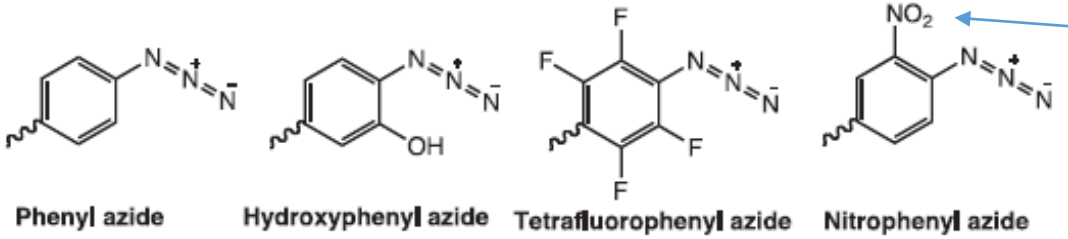
Succinimidyl-3-(2-pyridyldithio)propionate (**SPDP**)

-Water insoluble, thiol cleavable, membrane permeable
-NHS reacts with amine at pH 7-9 to form amide bond
-Pyridyl disulfide reacts with thiols at pH 6.5-7.5
-Protein thiolation and reversible protein–protein conjugation
-Attach haptens to carrier proteins for antibody production
-Preparation of immunotoxin conjugates
(Ref. 242-245)

Příklad thioly štěpitelného bifunkčního činidla

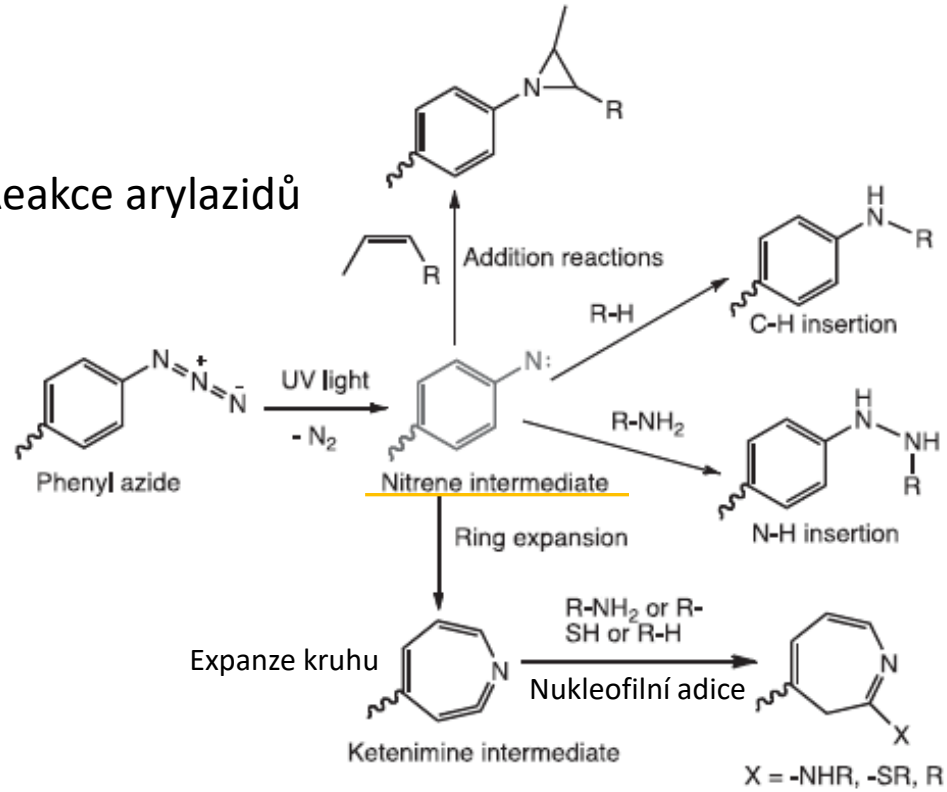
Fotoreaktivní „crosslinking“ činidla

Fotoreaktivní činidla jsou obvykle normálně chemicky inertní látky, které se stávají reaktivními po ozáření UV či VIS světlem.
Jsou využívána např. pro mapování interakcí mezi proteiny.
Tato činidla jsou velmi často na bázi *arylazidů*.



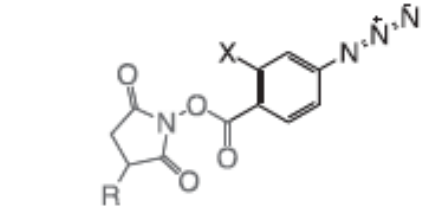
„Deaktivující“ nitro skupina posunuje fotoktivaci do delších vlnových délek (320-350 nm), méně škodlivých pro proteiny

Reakce arylazidů



Fotoaktivní látky jsou velmi reaktivní, nebezpečí vedlejších reakcí a deaktivace proteinů

Příklady heterobifunkčních aryl azidových fotoaktivních činidel s NHS-esterem (na aminy)



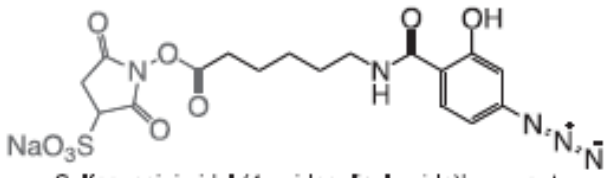
R = H, X = OH, N-hydroxysuccinimidyl-4-azidosalicylic acid (**NHS-ASA**)

R = SO_3Na, X = OH, N-hydroxysulfosuccinimidyl-4-azidosalicylic acid (**sulfo-NHS-ASA**)

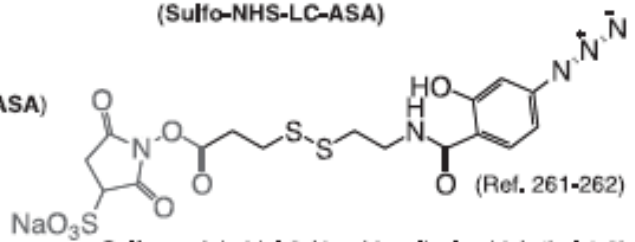
R = H, X = H, N-hydroxysuccinimidyl-4-azidobenzoate (**HSAB**)

R = SO_3Na, X = H, N-hydroxysulfosuccinimidyl-4-azidobenzoate (**sulfo-HSAB**)

-Used for photoaffinity labeling studies (Ref. 257-260)

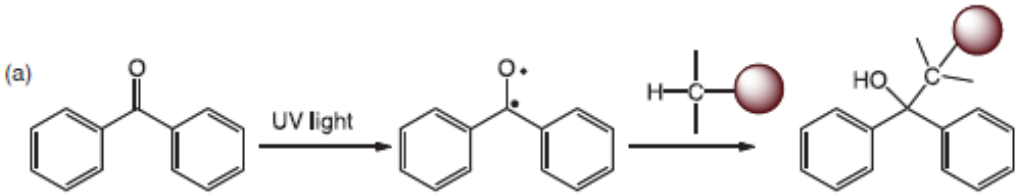


Sulfosuccinimidyl-(4-azidosalicylamido)hexanoate (**Sulfo-NHS-LC-ASA**)



Sulfosuccinimidyl-2-(4-azidosalicylamido)ethyl-1,3'-dithiopropionate (**SASD**) (Ref. 261-262)

Fotoreaktivní činidla na bázi benzofenonu



Benzophenone-4-maleimide

-water insoluble

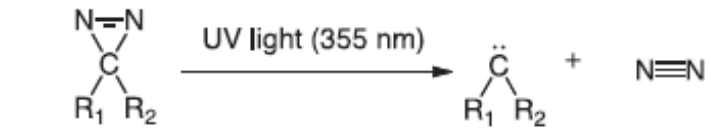
-Efficient irreversible protein cross-linking (Ref. 276-279)

Benzophenone-4-iodoacetamide

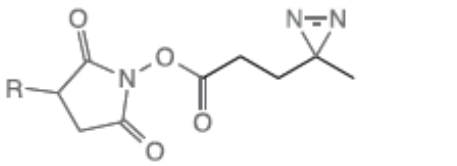
-water insoluble

-study of protein interactions (Ref. 280-281)

Příklady heterobifunkčních fotoaktivních činidel na bázi diazirinu

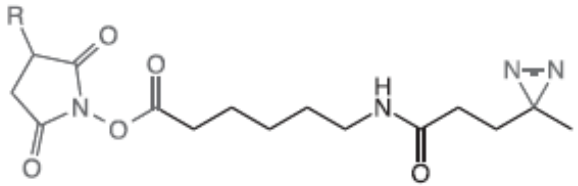


Reaguje např. s peptidovou vazbou



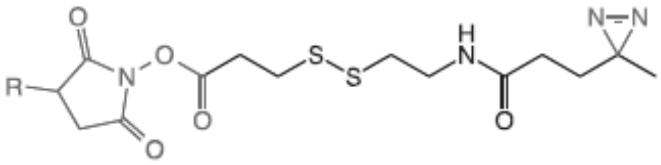
R = H, SDA (water insoluble)

R = SO_3Na, Sulfo SDA (water-soluble)



R = H, LC-SDA (water insoluble)

R = SO_3Na, Sulfo LC-SDA (water-soluble)



R = H, SDAD (water insoluble, cleavable)

R = SO_3Na, Sulfo SDAD (water-soluble, cleavable)

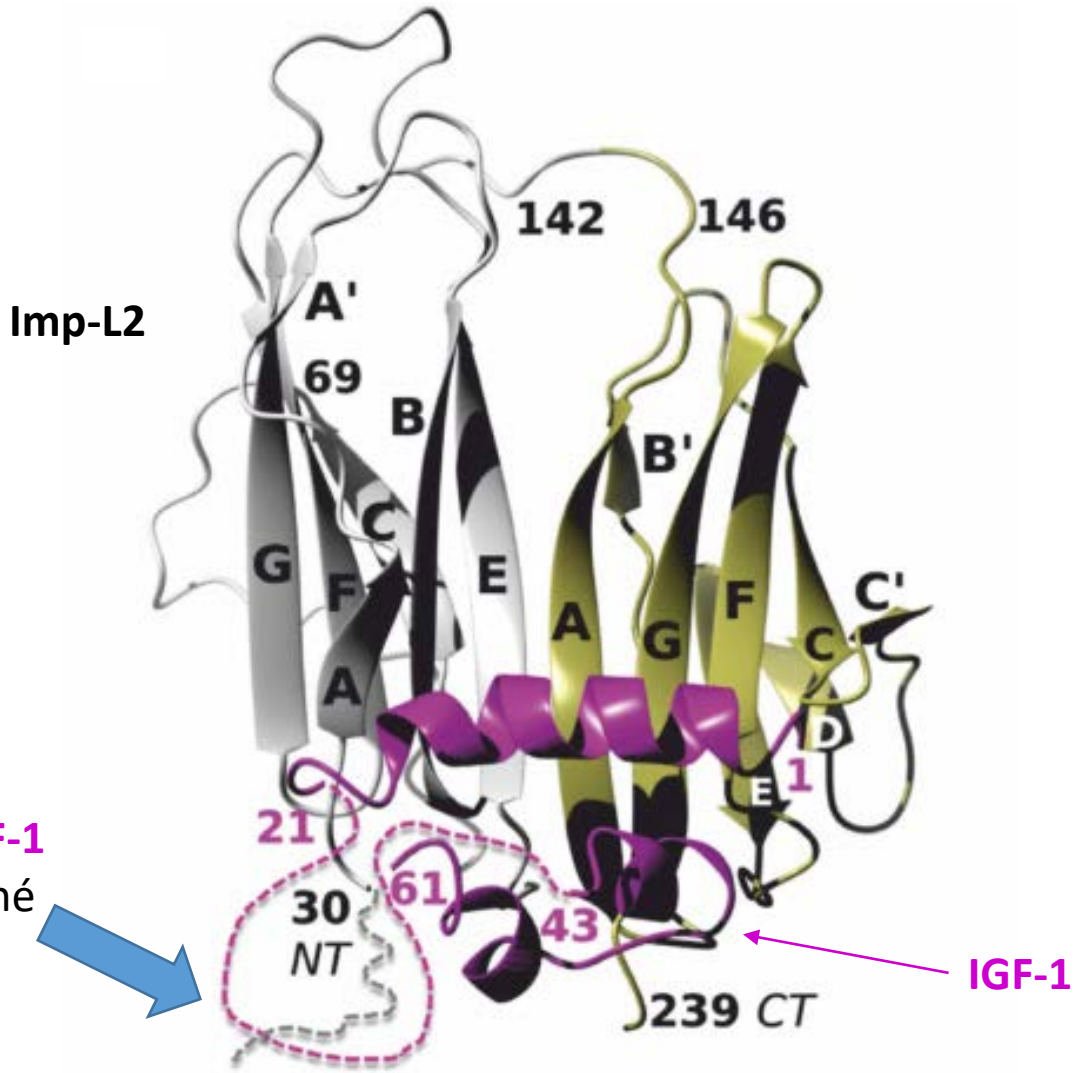
Cross-Linking/Mass Spectrometry Uncovers Details of Insulin-Like Growth Factor Interaction With Insect Insulin Binding Protein Imp-L2

Petr Pompach[1], Cristina M. Viola[2], Jelena Radosavljević[3†], Jingjing Lin[3], Jiří Jiráček[3], Andrzej M. Brzozowski[2] and Irena Selicharová[3]*

[1] Institute of Microbiology, Czech Academy of Sciences, Prague, Czechia, [2] York Structural Biology Laboratory, Department of Chemistry, The University of York, York, United Kingdom, [3] Institute of Organic Chemistry and Biochemistry, Czech Academy of Sciences, Prague, Czechia

Structural details of changes accompanying interaction between insulin-related hormones and their binding partners are often enigmatic. Here, cross-linking/mass spectrometry could complement structural techniques and reveal details of these protein-protein interfaces. We used such approach to clarify missing structural description of the interface in human insulin-like growth factor (IGF-1): *Drosophila melanogaster* imaginal morphogenesis protein-late 2 protein (Imp-L2) complex which we studied previously by X-ray crystallography. We crosslinked these proteins by heterobifunctional cross-linker sulfosuccinimidyl 4,4′-azidopentanoate (Sulfo-SDA) for the subsequent mass spectrometry (MS) analysis. The MS analysis revealed IGF-1:Imp-L2 interactions which were not resolved in the crystal structure of this assembly, and they converged with X-ray results, indicating the importance of the IGF-1 N-terminus interaction with the *C*-terminal (185–242) part of the Imp-L2 for stability of this complex. Here, we also showed the advantage and reliability of MS approach in solving details of protein-protein interactions that are too flexible for solid state structural methods.

Krystalová struktura



Aminokyseliny 22-42 z **IGF-1** nebyly v komplexu viditelné

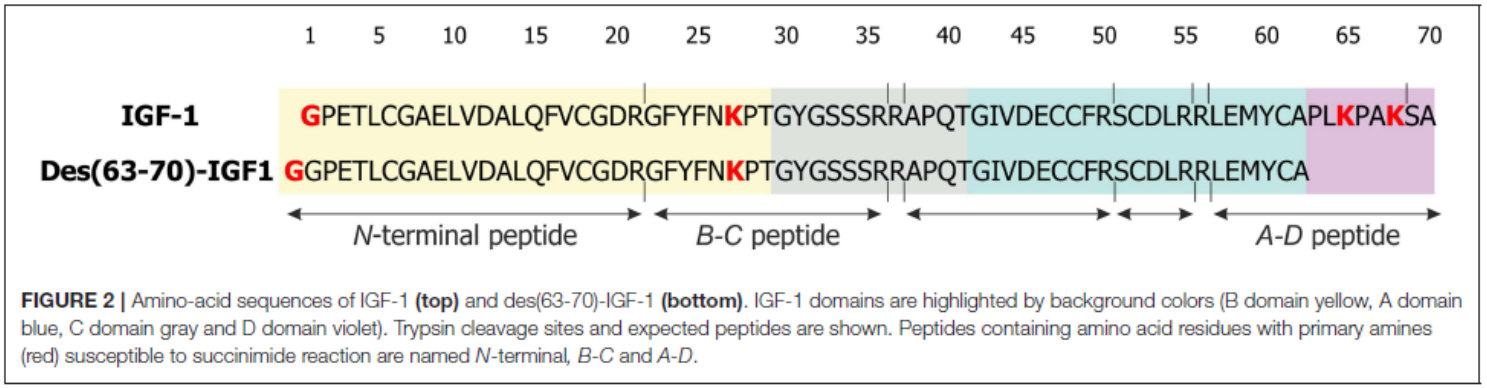
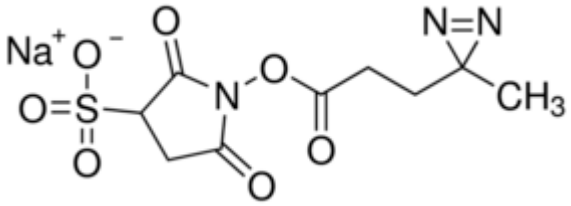
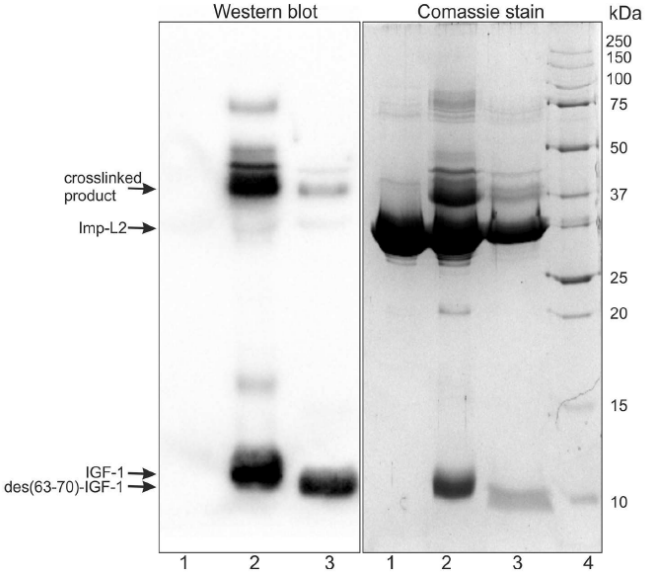
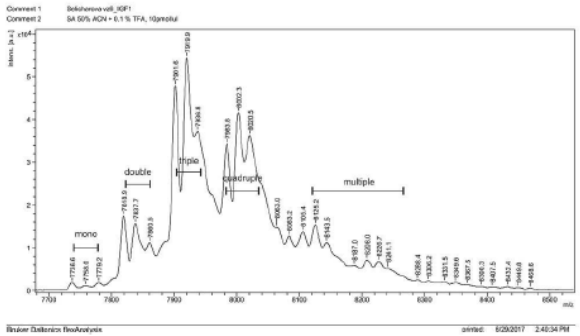


FIGURE 2 | Amino-acid sequences of IGF-1 **(top)** and des(63-70)-IGF-1 **(bottom)**. IGF-1 domains are highlighted by background colors (B domain yellow, A domain blue, C domain gray and D domain violet). Trypsin cleavage sites and expected peptides are shown. Peptides containing amino acid residues with primary amines (red) susceptible to succinimide reaction are named *N*-terminal, *B-C* and *A-D*.



- červené lysiny K byly označeny pomocí Sulfo-SDA
- byl znovu připraven komplex s vazebným proteinem
- pak se posvítilo při 365 nm
- produkty analyzovány pomocí SDS-Elfo a WB
- produkt vystřižen z gelu, štěpen trypsinem, analyzován MS



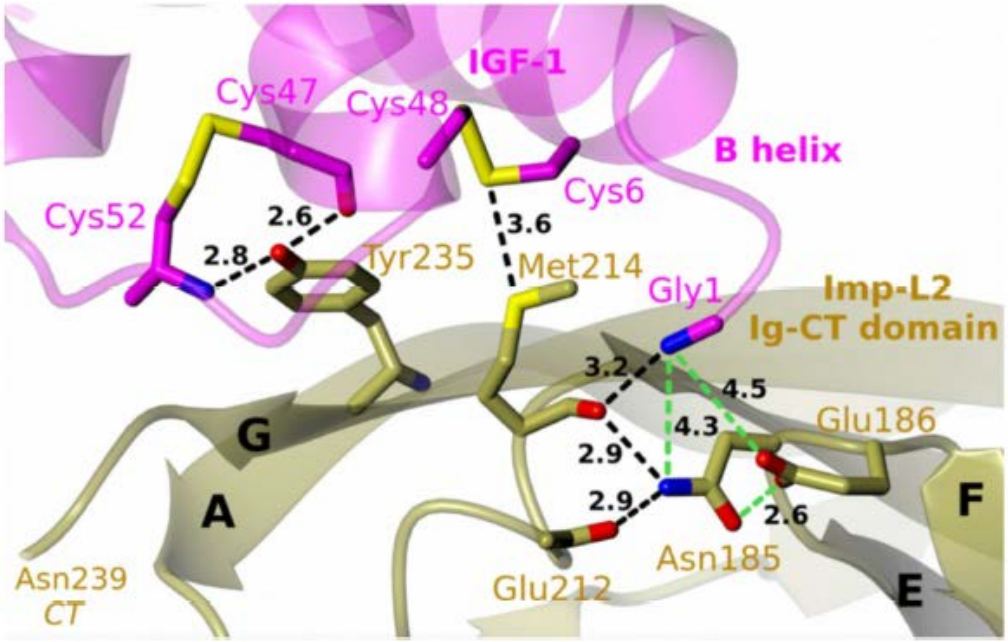


Crosslinked peptide (IGF-1)–(Imp-L2)	Exp. mass	Thr. mass	Error	StavroX score
(1, 21)–(178, 188)	3,719.7681	3719.7680	−0.04	79
(1, 21)–(211, 218)	3430.5381	3430.5388	−0.2	24
(1, 21)–(142, 150)	3405.6567	3405.6566	+0.03	61
(22, 36)–(2, 24)	4307.9859	4307.9851	+0.19	41

Nově objevené oblasti v Impl-2, které reagují s IGF-1

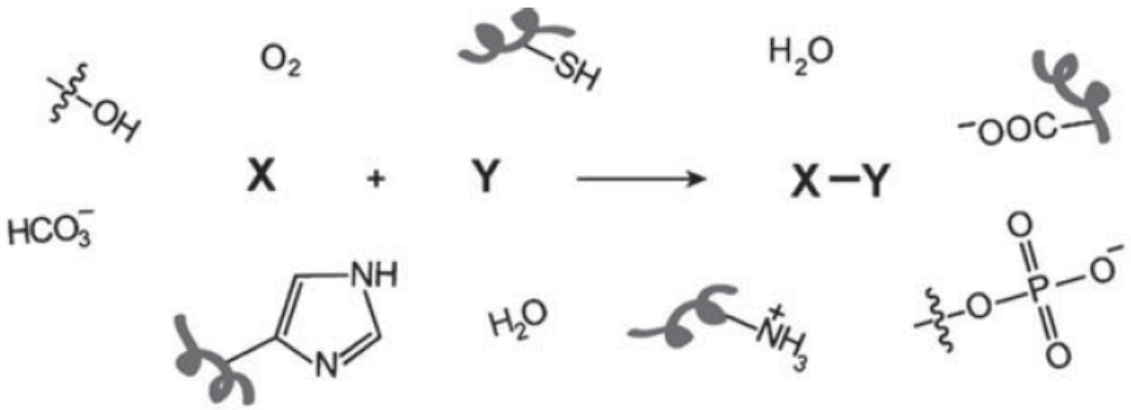
```
1          10         20         30         40         50         60
RAVDLVDDSNDVDNSIEAEEEKPRNRAFEADWLKFTKTPPTKLQQADGATIEIVCEMMGS

           70         80         90         100        110        120
QVPSIQWVVGHLPRSELDDLDSNQVAEEAPSAIVRVRSSHIIDHVLSEARTYTCVGRTGS

           130        140        150        160        170        180
KTIYASTVVHPPRSSRLTPEKTYPGAQKPRIIYTEKTHLDLMGSNIQLPCRVHARPRAEI

           190        200        210   * *  220        230   * ** *240
TWLNNENKEIVQGHRHRVLANGDLLISEIKWEDMGNYKCIARNVVGKDTADTFVYPVLNEED
```

Modelování nově objevených interakcí

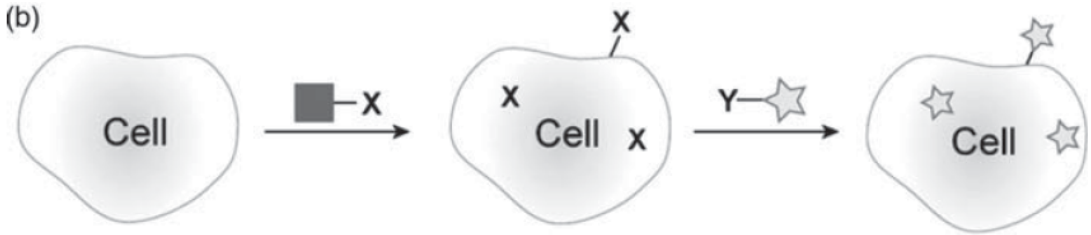


Bio-ortogonální reakce

Takové reakce, které selektivně probíhají v živých systémech bez toho, aby interferovaly s biologickými procesy.

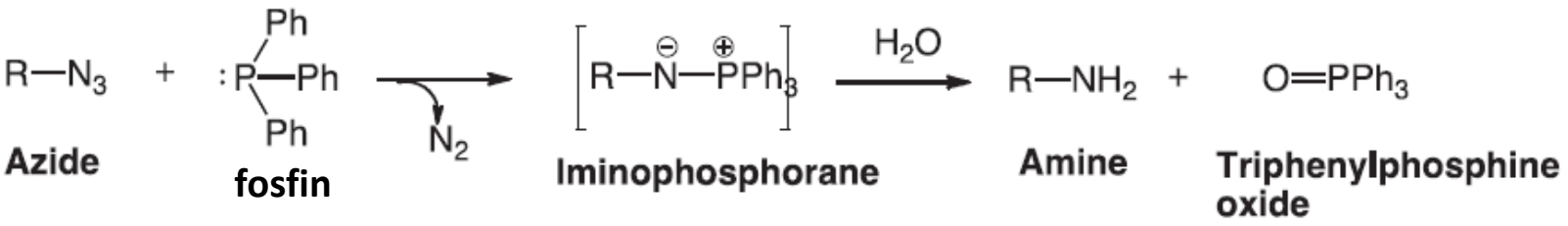


Obvykle je do biologického objektu nejprve vnesena specifická skupina X, která pak selektivně reaguje s jinou specifickou skupinou Y.



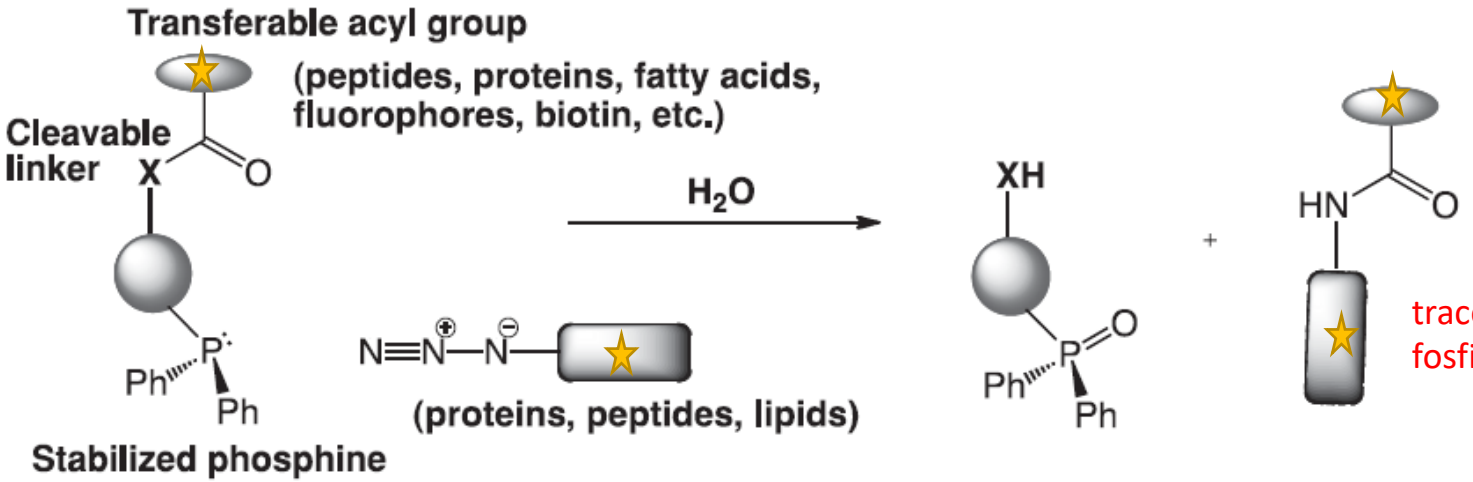
Staudingerova ligace

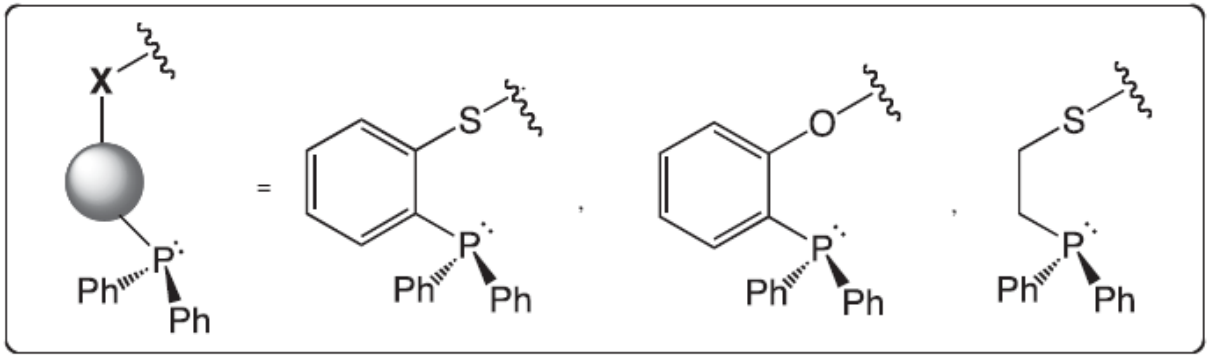
1919, Staudinger a Meyer objevili Staudingerovu reakci, vlastně redukci azidu na amin fosfinem



V roce 2000 Carolyn A. Bertozzi modifikovala Staudingerovu reakci tak, že je ligací tzv. „Traceless“ bio-orthogonal Staudinger ligation

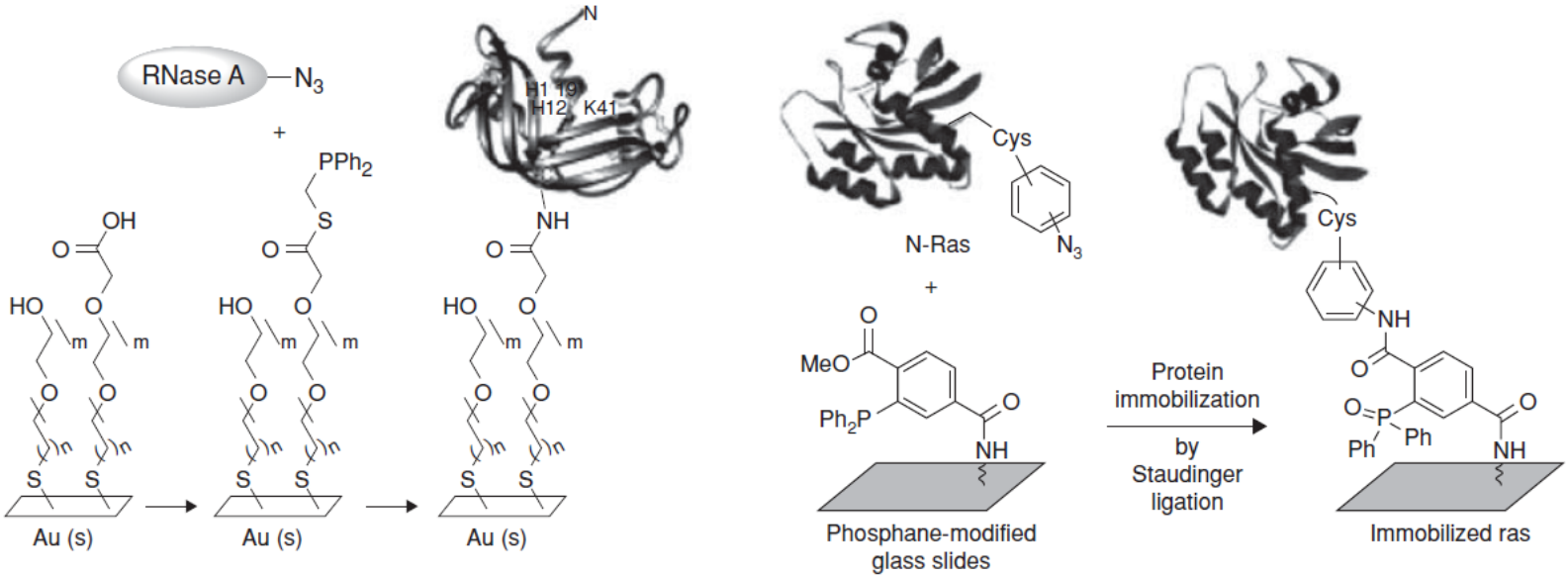
Azidy ani fosfiny se v přírodě nevyskytují, musí se ale do biomolekul tedy zavést





1. redukce azidu na amin a 2. nukleofilní atak (thio)esteru

Aplikace Staudingerovy ligace (modifikace povrchů)



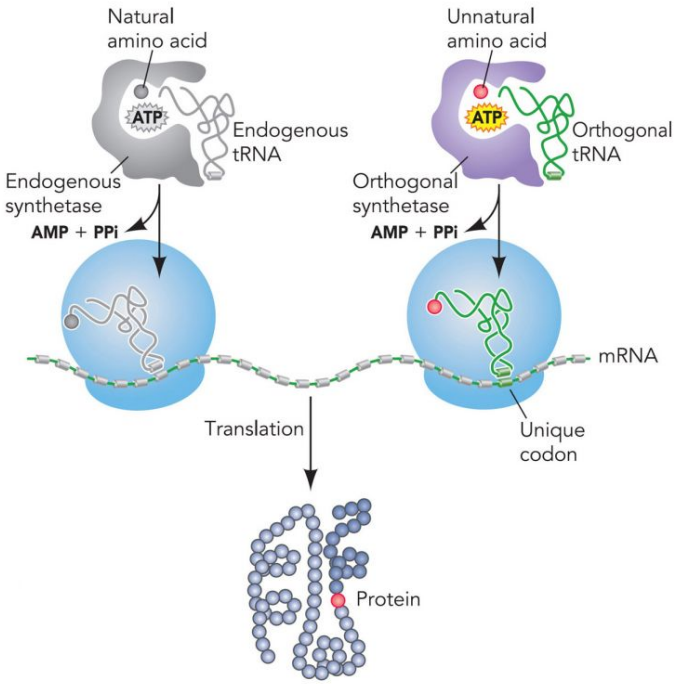
Nevýhodou je značná pomalost reakce a tendence fosfinů oxidovat se.

Ale jak dostat do proteinu azidoskupinu?

Expandovaný genetický kód

Tato metoda umožňuje vnášet nekódované aminokyseliny do proteinů během normální rekombinantní přípravy.

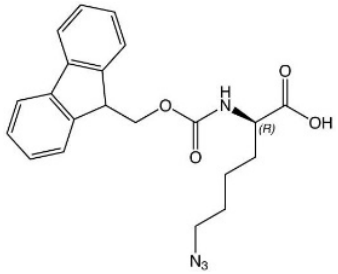
Schultz, Peter. G. (2001). "Expanding the Genetic Code of Escherichia coli". Science 292 (5516): 498–500.



„To this end, we have developed a methodology that allows one to genetically encode novel amino acids, beyond the common twenty, in both prokaryotic and eukaryotic organisms. This methodology involves the generation a unique codon-tRNA pair and corresponding aminoacyl-tRNA synthetase. Specifically, an orthogonal tRNA is constructed that is not a substrate for any natural aminoacyl synthetases and which inserts its cognate amino acid into the growing polypeptide chain in response to a nonsense or frameshift codon. A cognate synthetase is then generated which recognizes this unique tRNA and no other; the substrate specificity of this synthetase is then evolved to recognize a desired "twenty first" amino acid, and no endogenous amino acid. We have shown that this methodology can be used to efficiently incorporate a large number of unnatural amino acids into proteins in *E. coli*, yeast and mammalian cells with fidelity and efficiency rivaling that of the common 20 amino acids."

http://schultz.scripps.edu/main.php

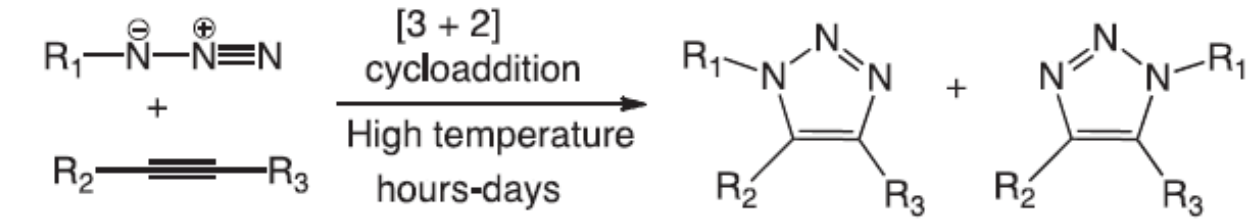
Anebo syntéza na pevné fázi s Fmoc-azidoaminokyselinou



Click chemie

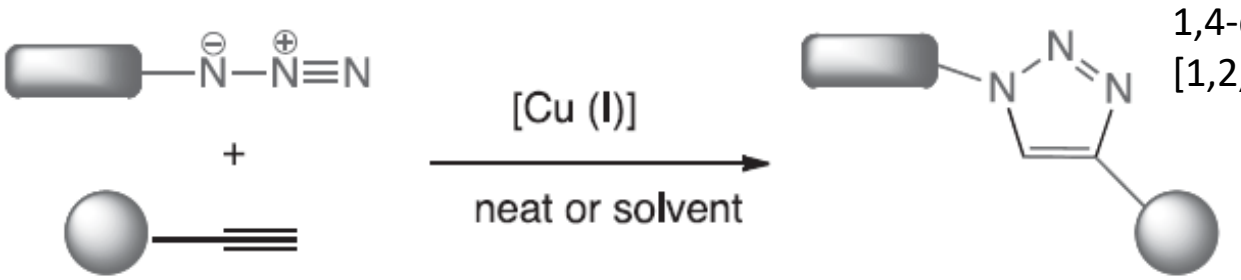
(Copper(I)-catalyzed Azide-Alkyne Cycloaddition, CuAAC)

Její princip je založen na tzv. Huisgenově 1,3-dipolární cykloadici,
která ovšem probíhá pomalu a není regioselektivní



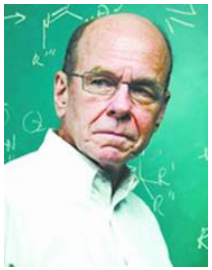
R_1, R_2, R_3 = alkyl, aryl

V roce 2002, Meldal a Sharpless nezávisle objevili, že reakce mezi azidy a terminálními alkyny
je až 10^6 x urychlena přítomností $Cu^{(1+)}$



1,4-disubstituované
[1,2,3]-triazoly

Tato dnes extrémně populární reakce probíhá dobře takřka ve všech prostředích.



Karl Barry Sharples

Nositel Nobelovy ceny za chemii za rok 2001.
Obdržel ji za objev oxidačních reakcí vytvářejících asymetrické chirální aminokyseliny (DOPA).

A Stepwise Huisgen Cycloaddition Process: Copper(I)-Catalyzed Regioselective "Ligation" of Azides and Terminal Alkynes**

Vsevolod V. Rostovtsev, Luke G. Green,
Valery V. Fokin,* and K. Barry Sharpless*

Angew. Chem. Int. Ed. **2002**, *41*, No. 14, 2596

Morten P. Meldal (born 1954 in Denmark) is a Danish chemist.

J. Org. Chem. **2002**, *67*, 3057−3064

Peptidotriazoles on Solid Phase: [1,2,3]-Triazoles by Regiospecific Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions of Terminal Alkynes to Azides

Christian W. Tornøe, Caspar Christensen, and Morten Meldal*

Peptides: The Wave of the Future
Michal Lebl and Richard A. Houghten (Editors)
American Peptide Society, 2001

Peptidotriazoles: Copper(I)-Catalyzed 1,3-Dipolar Cycloadditions on Solid-Phase

Christian W. Tornøe and Morten Meldal

Center for Solid Phase Organic Combinatorial Chemistry, Department of Chemistry, Carlsberg Laboratory, DK-2500 Valby, Denmark

Excelentní review

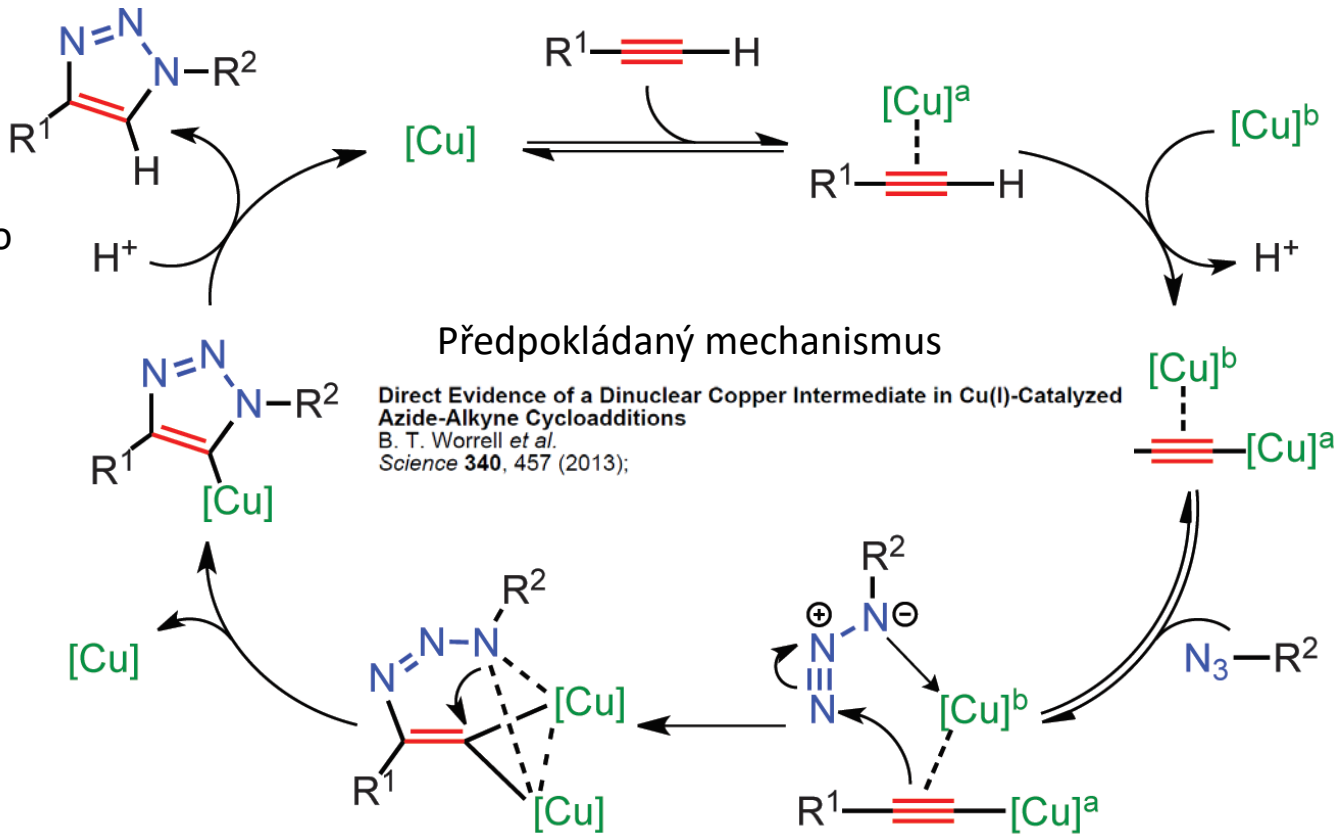
Chem. Rev. **2008**, *108*, 2952–3015

Cu-Catalyzed Azide–Alkyne Cycloaddition

Morten Meldal*,† and Christian Wenzel Tornøe‡

Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark, and H. Lundbeck A/S, Ottiliavej 9, DK-2500 Valby, Denmark

Stejná Mr jako reaktanty

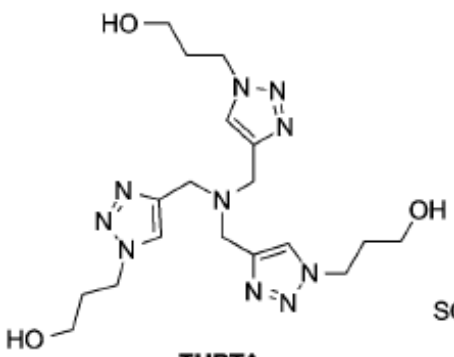


Předpokládaný mechanismus

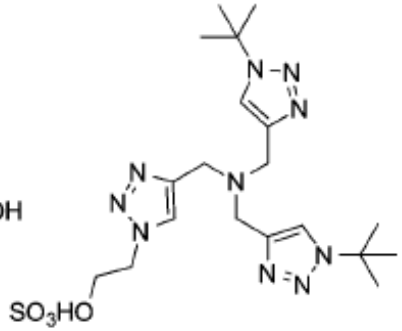
Direct Evidence of a Dinuclear Copper Intermediate in Cu(I)-Catalyzed Azide-Alkyne Cycloadditions
B. T. Worrell *et al.*
Science **340**, 457 (2013);

Měď se teoreticky recykluje (ale snadno oxiduje se na Cu^{2+}). Zůstává zachován stejný počet atomů. Jen terminální alkyny.

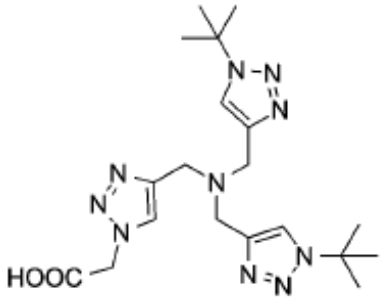
Látky urychlující CuAAC



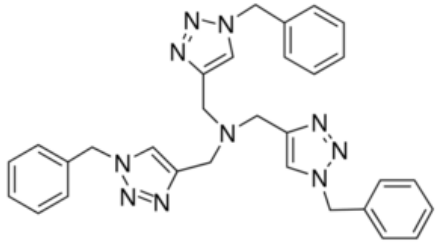
THPTA



BTTES



BTTAA



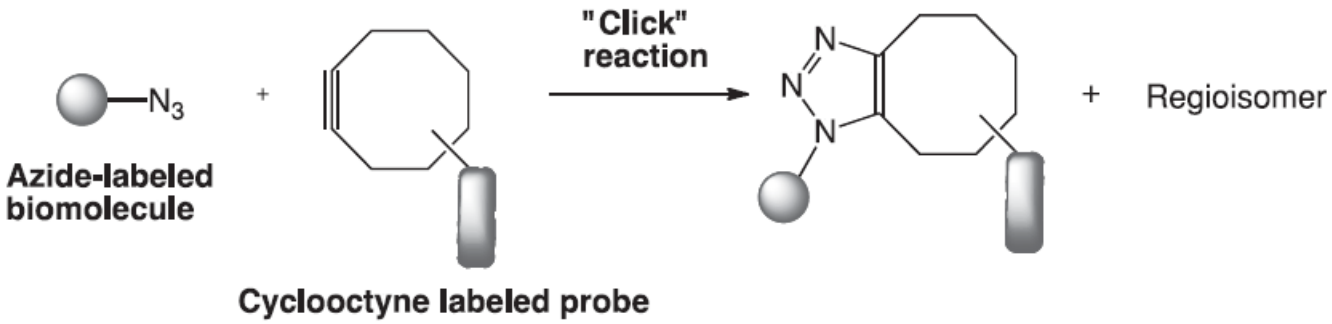
TBTA

Koordinují a stabilizují $Cu^{(I)}$ a prý tak urychlují reakce

Click chemie bez mědi

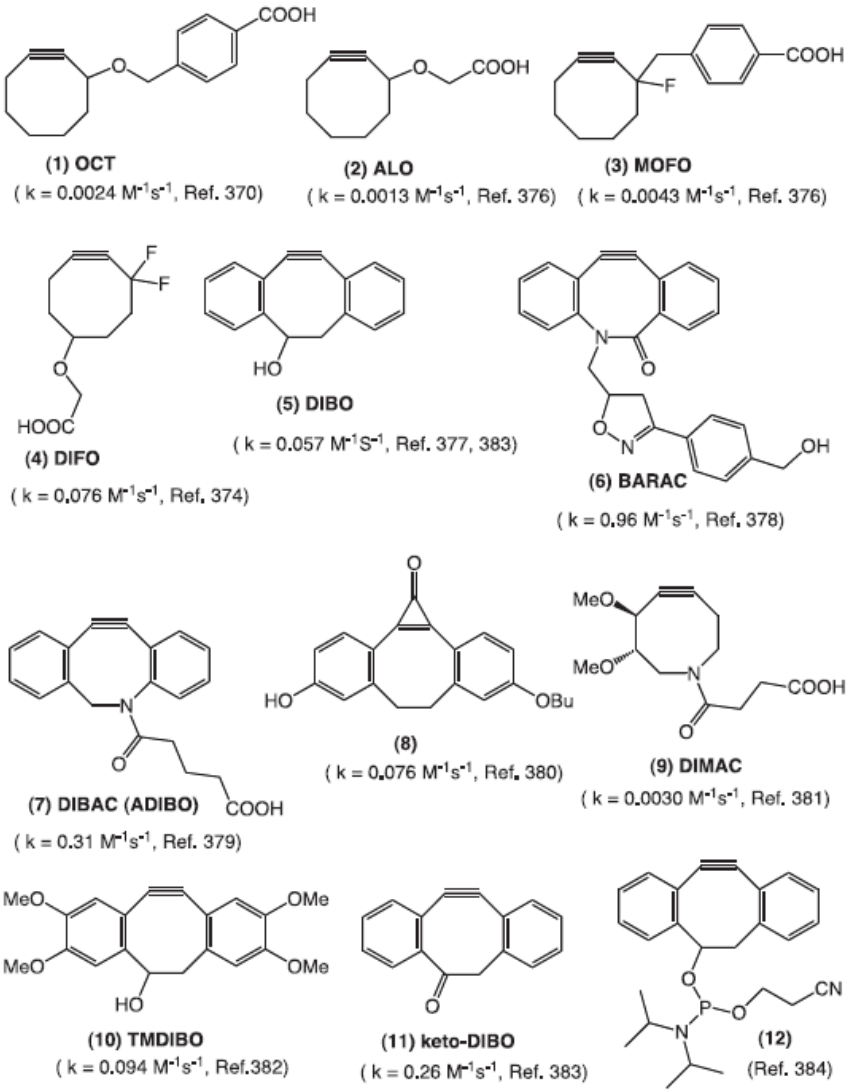
(Copper-free click chemistry)

Měď je toxická a snadno se chelatuje na peptidy.
Carolyne A. Bertozzi vyvinula alternativní click reakci bez katalýzy mědí (2010).



Reakce je ale pomalejší než při katalýze mědí.

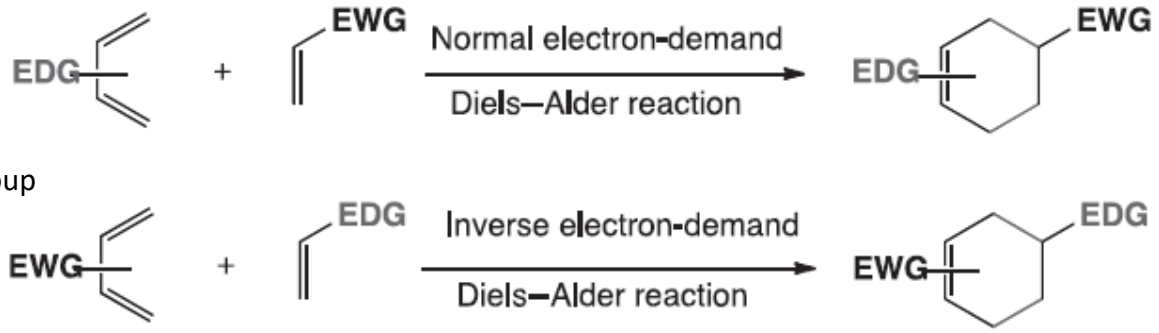
Nové cyklooktyny pro urychlení click reakce bez mědi

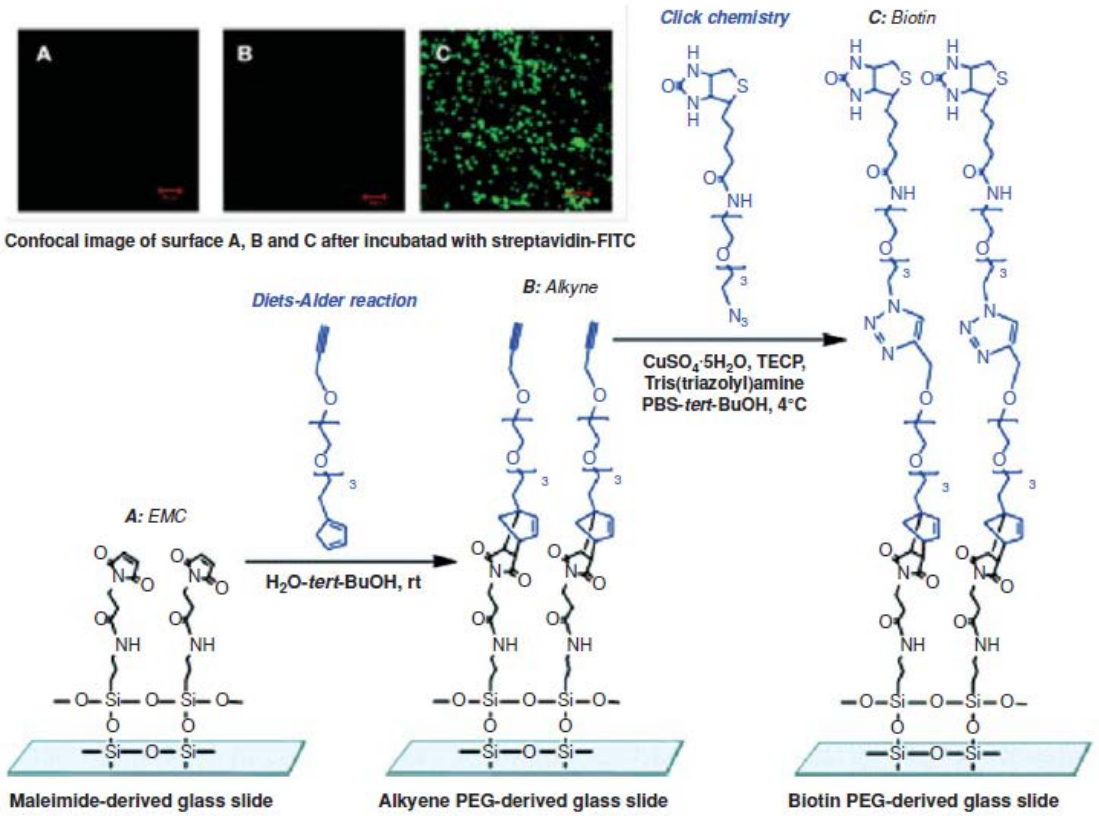


Bio-ortogonální reakce prostřednictvím alkenů

Diels-Adlerova cykloadice, [4+2]cykloadice

EDG je „electron-donating group“
EWG je „elekctron withdrawing“ group





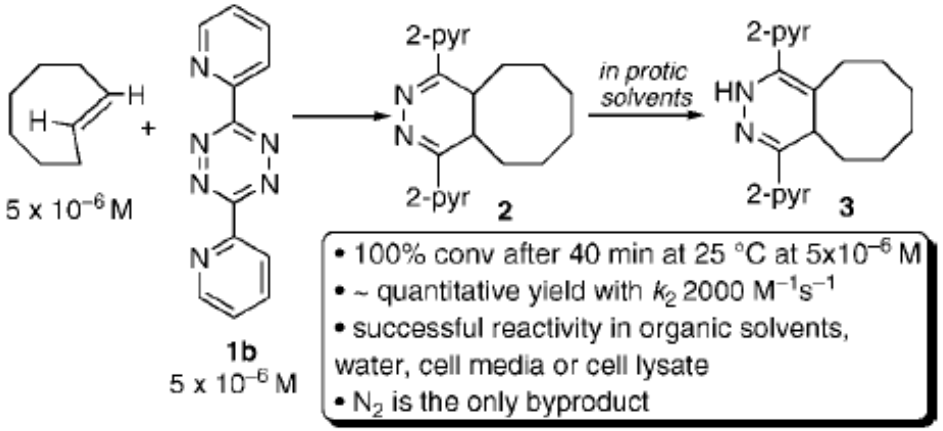
Cykloadice pomocí tetrazinů

„Inverse electron demand Diels-Alder“

Tetrazine Ligation: Fast Bioconjugation Based on Inverse-Electron-Demand Diels–Alder Reactivity

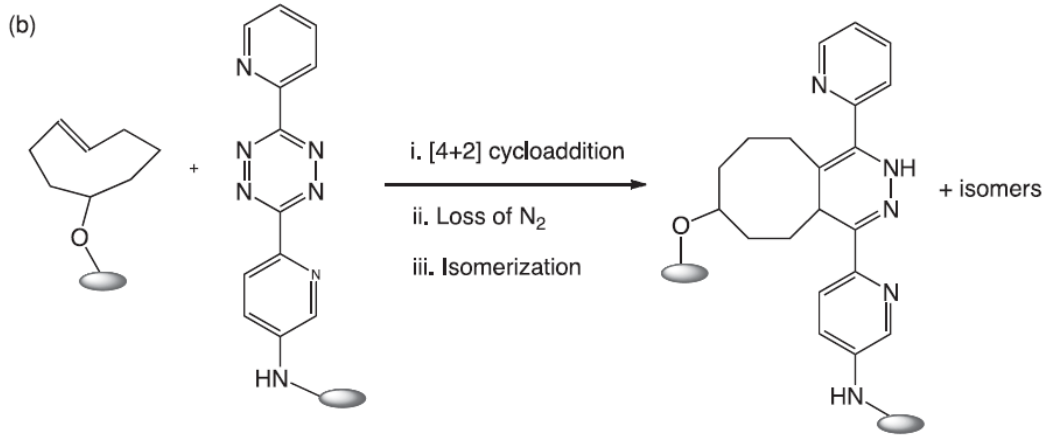
Melissa L. Blackman, Maksim Royzen, and Joseph M. Fox*

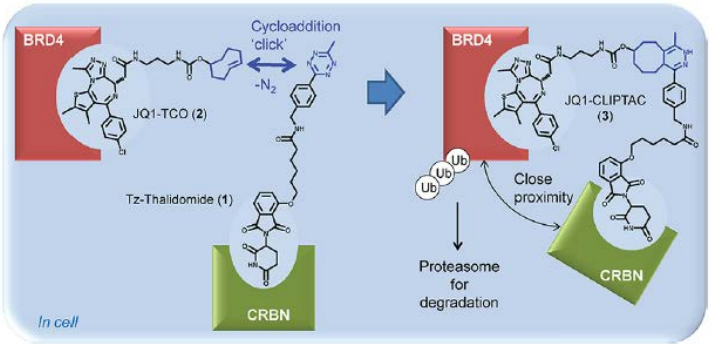
J. AM. CHEM. SOC. 2008, *130*, 13518–13519



velmi rychlé

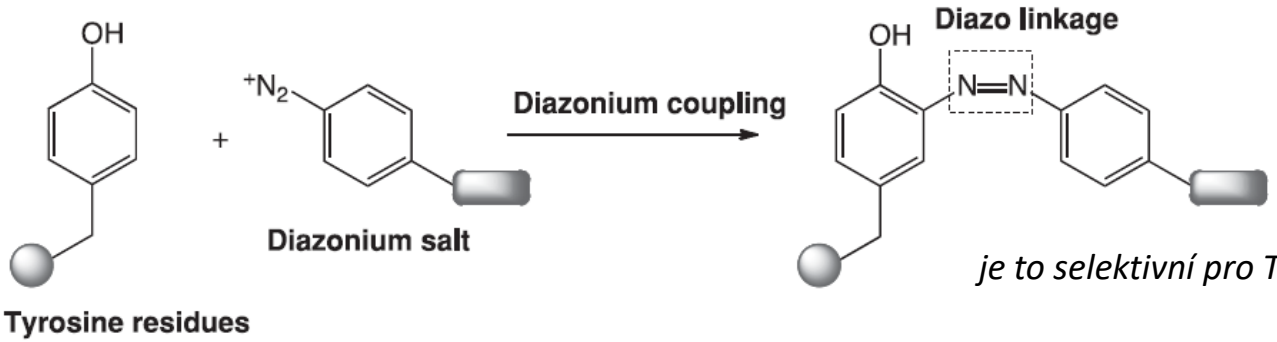
aplikace pro ligaci, dvou biomolekul





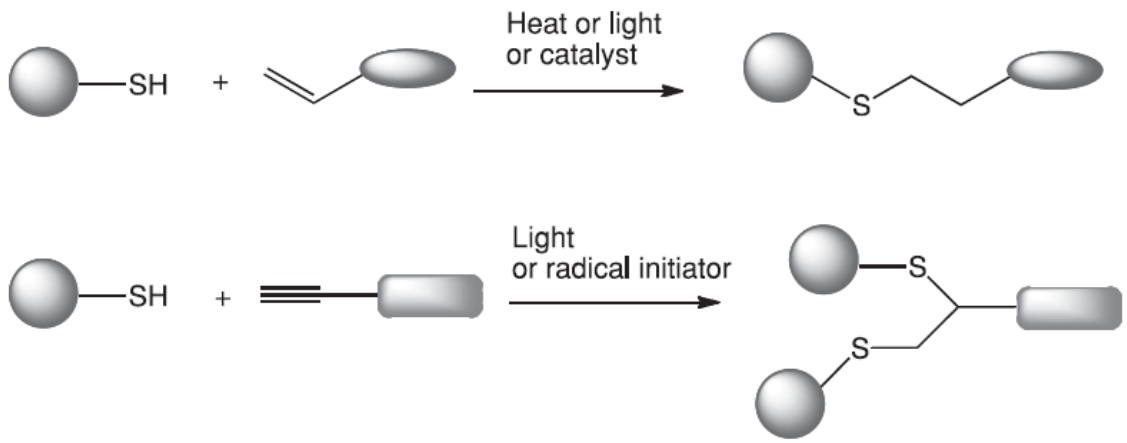
konjugace zapříčinila degradaci BRD4

Konjugace pomocí diazoniových solí



je to selektivní pro Tyr a His

Konjugace thiol-enovou a thiol-ynovou adicí (Michaelovy adice)

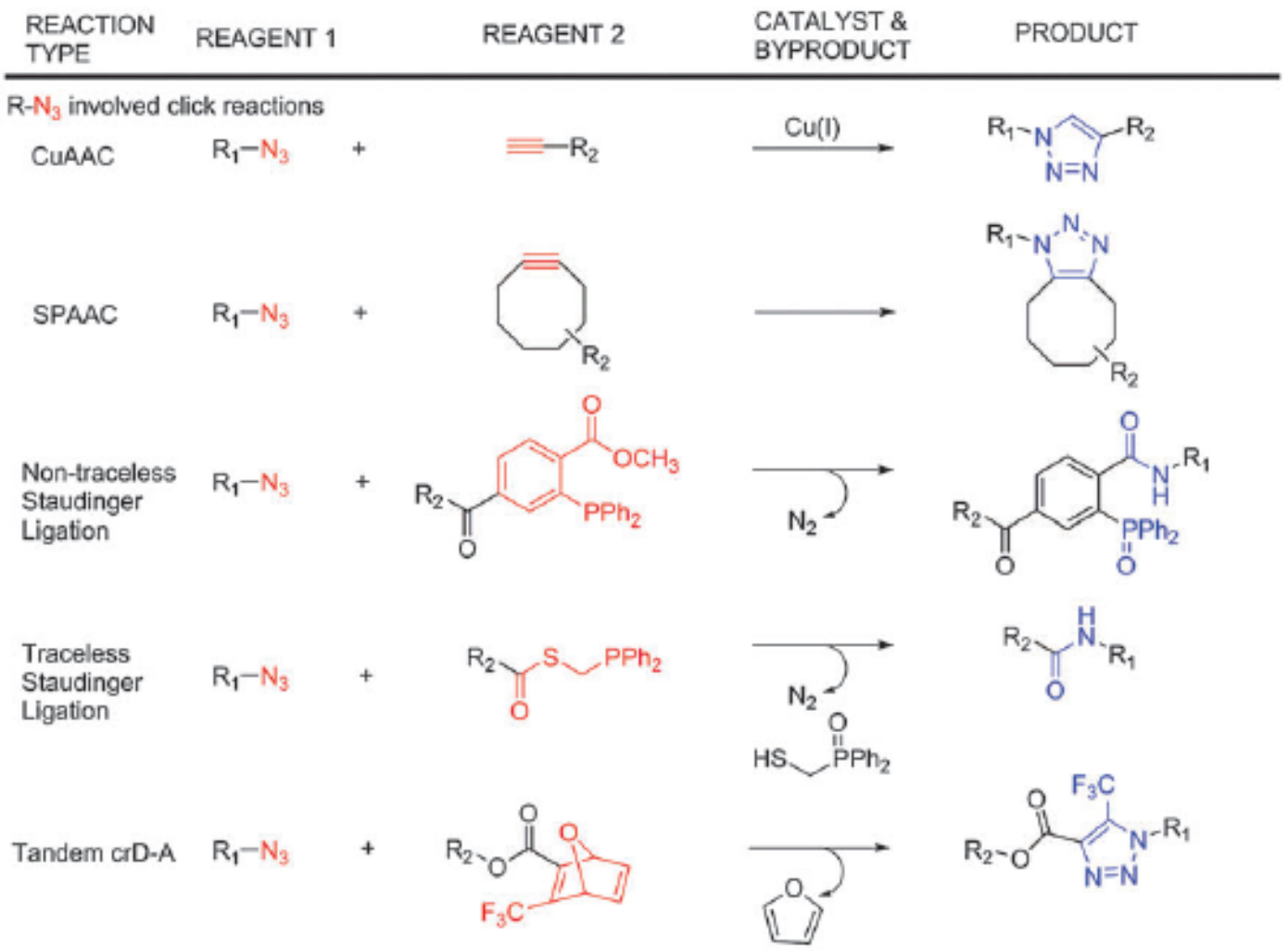


Přehled bio-ortogonálních „click“ reakcí používaných v peptidové chemii

Cite this: *Chem. Soc. Rev.*, 2014, 43, 7013

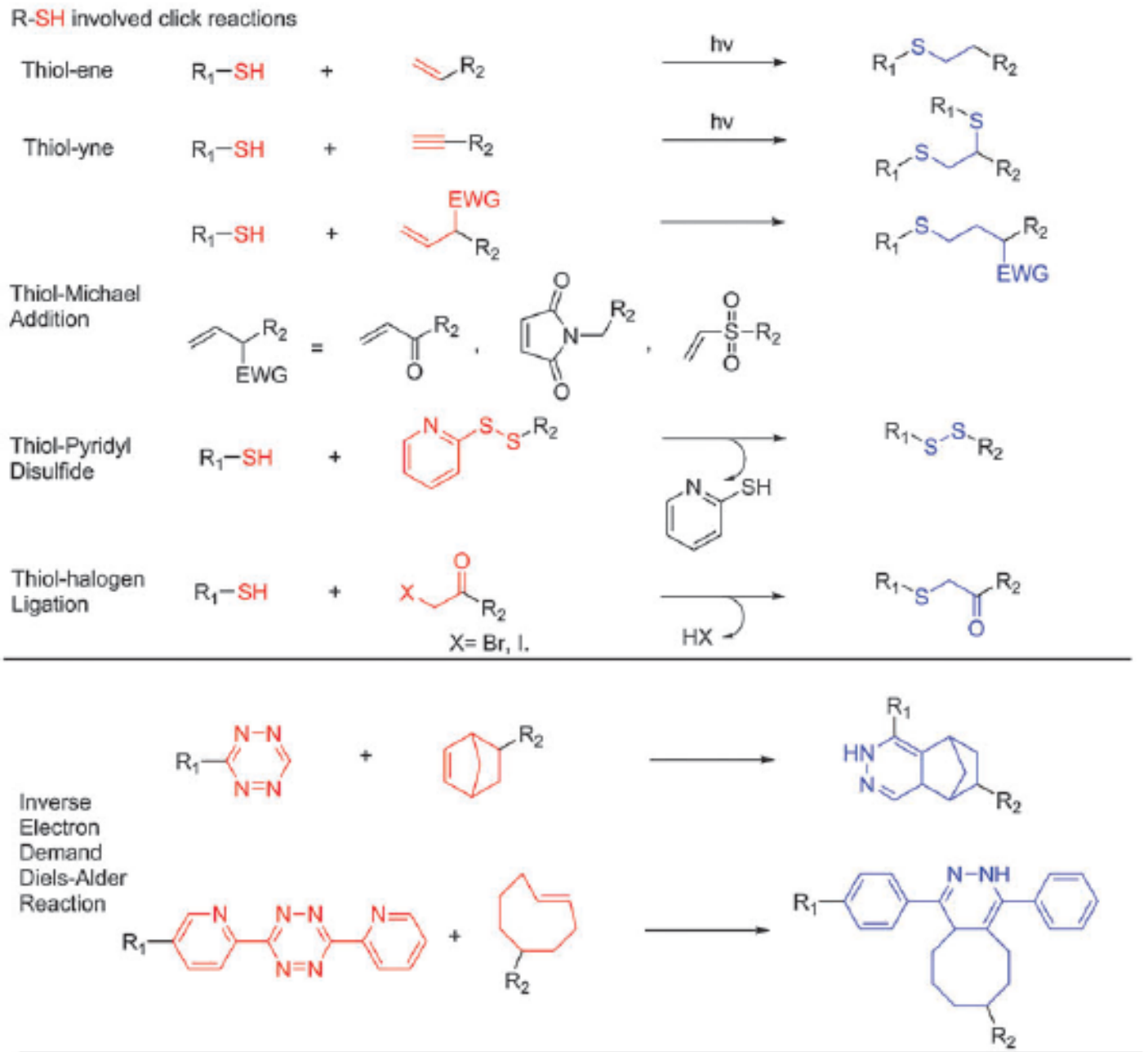
"Click" reactions: a versatile toolbox for the synthesis of peptide-conjugates

Wen Tang[a] and Matthew L. Becker*[ab]

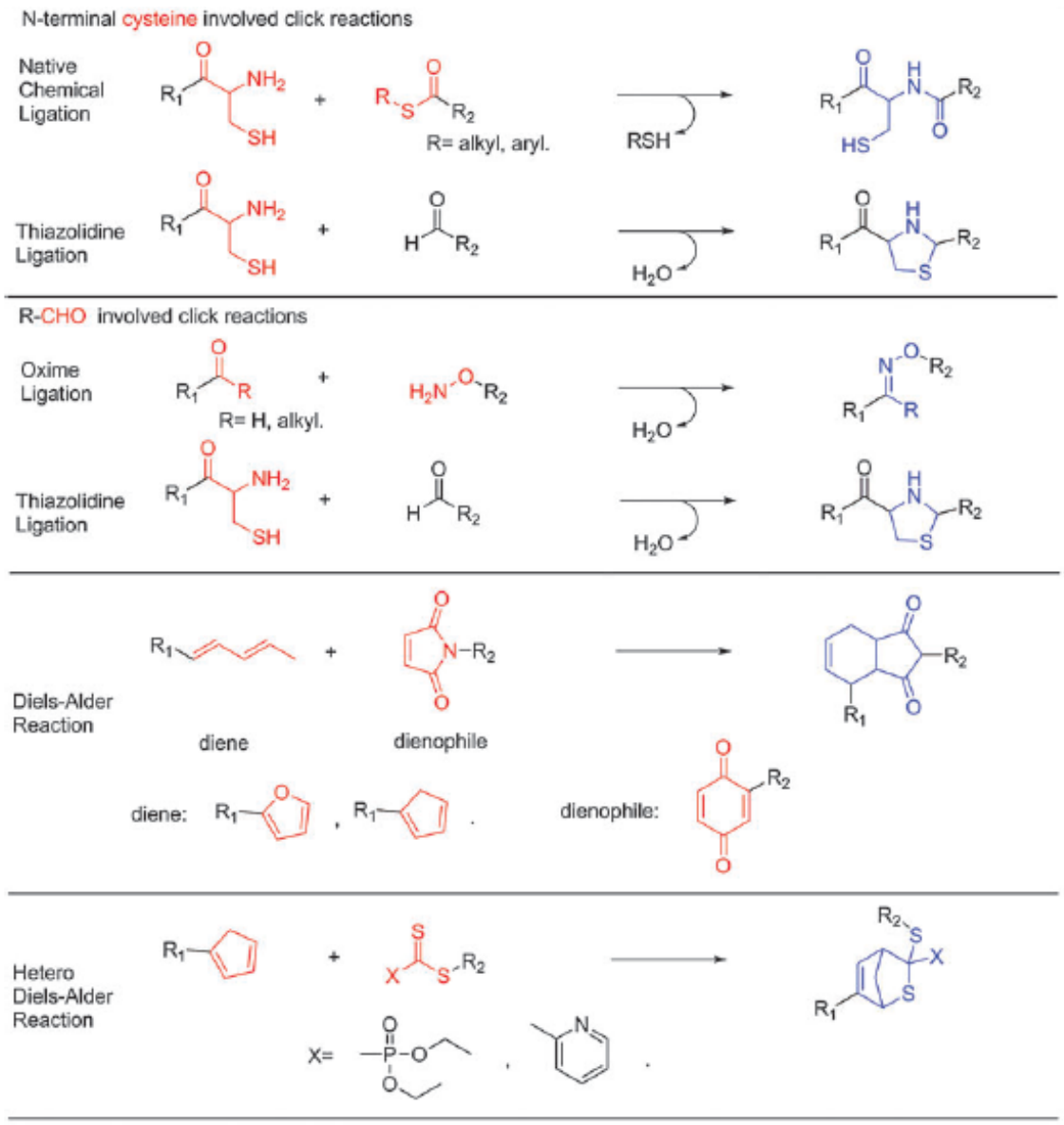


(Strain-promoted AAC)

Pokračování přehledu „click“ reakcí



Pokračování přehledu „click“ reakcí



Přehled „click“ reakcí mezi obvykle používanými funkčními skupinami

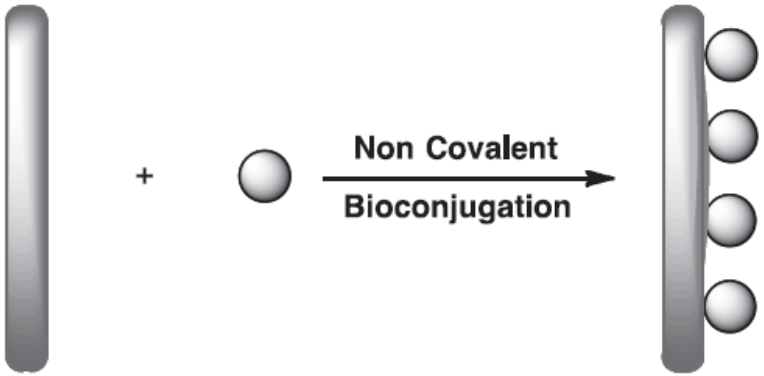
	$R-N_3$				R–SH		R–CHO	$R-ONH_2$						
$R-N_3$	--	--	Cu(I): CuAAC	SPAAC	--	--	--	--	--	--	--	--	Staudinger Ligation	--
	--	--	--	--	*hv*: Thiol-ene	*hv*: Thiol-ene	--	--	--	--	--	--	--	--
	Cu(I): CuAAC	--	--	--	*hv*: Thiol-yne	*hv*: Thiol-ene	--	--	--	--	--	--	--	--
	SPAAC	--	--	--	Thiol-yne	Thiol-yne	--	--	--	--	--	IEDA Reaction	--	--
R–SH	--	*hv*: Thiol-ene	*hv*: Thiol-yne	Thiol-yne	--	--	--	--	Thiol-Michael Addition	*hv*: Thiol-ene	*hv*: Thiol-ene	--	Thioester Exchange	Thiol-Pyridyl Difulfide
	--	*hv*: Thiol-ene	*hv*: Thiol-yne	Thiol-yne	--	--	pH=4-5: Thiazolidine Ligation	--	Thiol-Michael Addition	*hv*: Thiol-ene	hv: Thiol-ene	--	Thioester Exchange	Thiol-Pyridyl Difulfide
R–CHO	--	--	--	--	--	pH=4-5: Thiazolidine Ligation	--	pH 4.5: Oxime Ligation	--	--	--	--	--	--
$R-ONH_2$	--	--	--	--	--	--	pH 4.5: Oxime Ligation	--	--	--	--	--	--	--
	--	--	--	--	Thiol-Michael Addition	Thiol-Michael Addition	--	--	--	--	D-A Reaction	--	--	--
	--	--	--	--	*hv*: Thiol-ene	*hv*: Thiol-ene	--	--	--	--	--	IEDA Reaction	--	--
	--	--	--	--	*hv*: Thiol-ene	*hv*: Thiol-ene	--	--	D-A Reaction	--	--	--	--	--
	--	--	--	IEDA Reaction	--	--	--	--	--	IEDA Reaction	--	--	--	--
	Staudinger Ligation	--	--	--	Thioester Exchange	Thioester Exchange	--	--	--	--	--	--	--	--
	--	--	--	--	Thiol-Pyridyl Difulfide	Thiol-Pyridyl Difulfide	--	--	--	--	--	--	--	--

[a] Unless otherwise mentioned, the reaction condition is able to proceed in physiological condition. The required catalyst or light irradiation is noted before reaction type. [b] The abbreviation for reactions are as following: Cu-catalyzed azide-alkyl cycloaddition (CuAAC), string-promoted azide-alkyl cycloaddition (SPAAC), Diels–Alder (DA) reaction, and inverse-electron-demand Diels–Alder (IEDA) reaction.

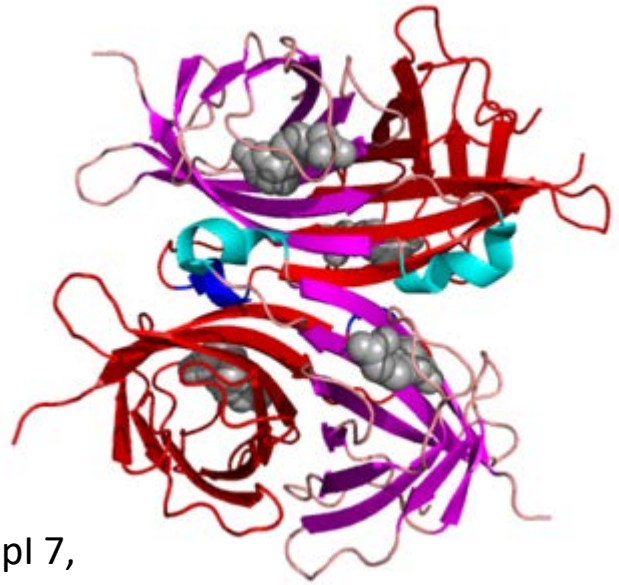
Nekovalentní biokonjugace

Nekovalentní interakce: elektrostatické (iontové, vodíkové, vazby halogenů), van der Waalsovy, Π-vazby, hydrofobní.

Nekovalentní vazby jsou relativně slabé (cca 0-60 KJ/mol), ale zase jich bývá hodně a může se pomocí nich obejít obtížná chemie.



Systém biotin-(strept)avidin

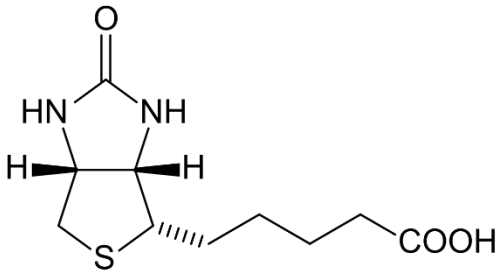


Avidin je tetramerní bazický glykoprotein (64 kDa) z *bílku ptáků* o pI 10. Výborně rozpustný ve vodě a stabilní v širokém rozmezí pH a teploty.

Streptavidin je glykoprotein (53 kDa) ze *Streptomyces avidinii* o pI 5 a je méně rozpustný.

Pierce prodává rekombinantní deglykosylovaný **Neutravidin** o pI 7, který neváže lektiny.

Avidiny mají extrémně vysokou afinitu vůči **biotinu** (vitamin B7 či H), K_d okolo 10^{-15}-10^{-13} M^{-1}.

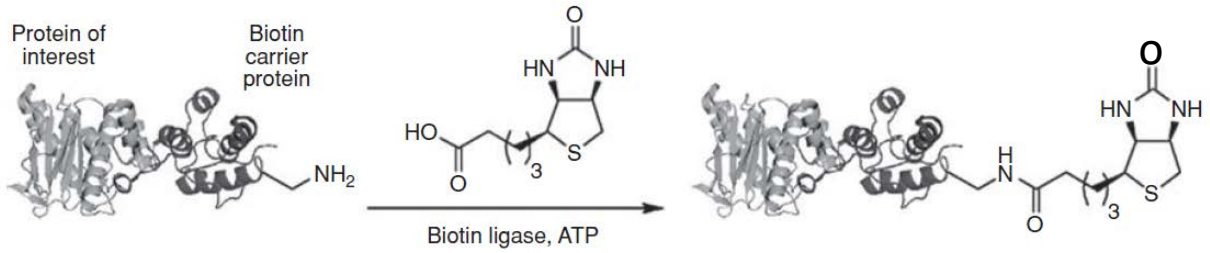


Interakce avidin-biotin se stala populární metodou pro imobilizaci proteinů na různé povrchy.

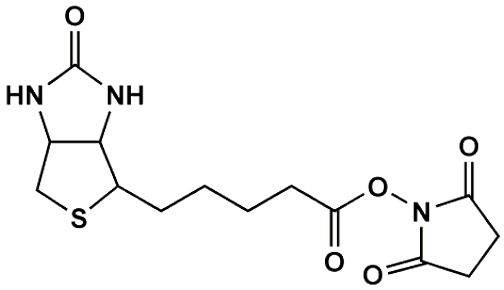
Je k dispozici řada komerčně dostupných povrchů potažených avidinem.

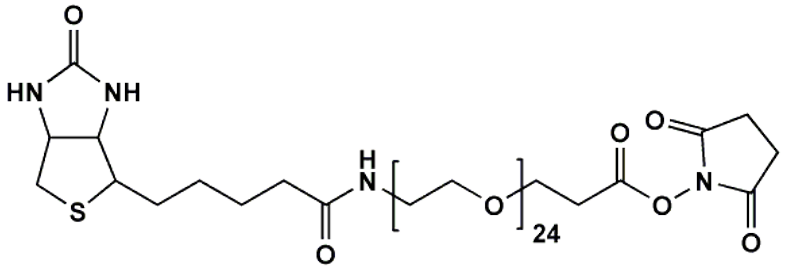
Je ale nutno označit biotinem protein, který má být imobilizován.

Pomocí biotin ligázy (BirA) z E. coli, která značí lysiny



Pomocí biotin NHS esterů, komerčně dostupný



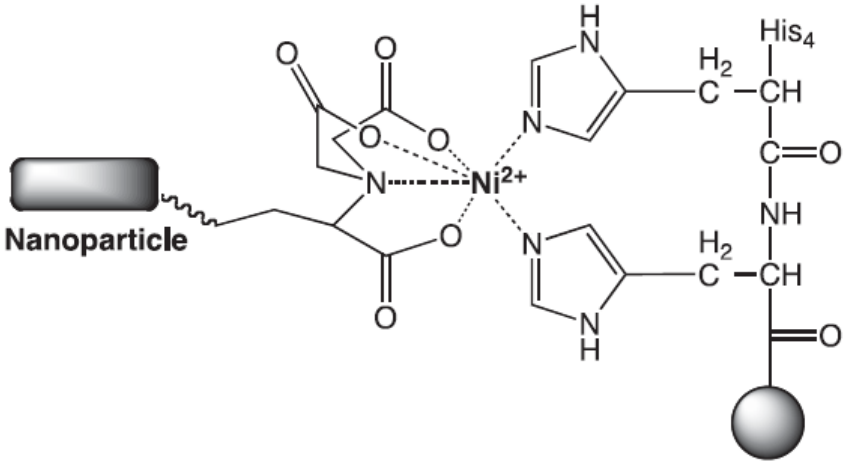


Systém zprostředkovaný konjugací pomocí kovových iontů

Organometallic–Peptide Bioconjugates: Synthetic Strategies and Medicinal Applications

Bauke Albada*,† and Nils Metzler-Nolte*,‡

DOI: 10.1021/acs.chemrev.6b00166
Chem. Rev. 2016, 116, 11797–11839



poly-histidinový motiv chelatující kovy

His-tag je polyhistidinová sekvence sloužící k afinitní purifikaci rekombinantních proteinů

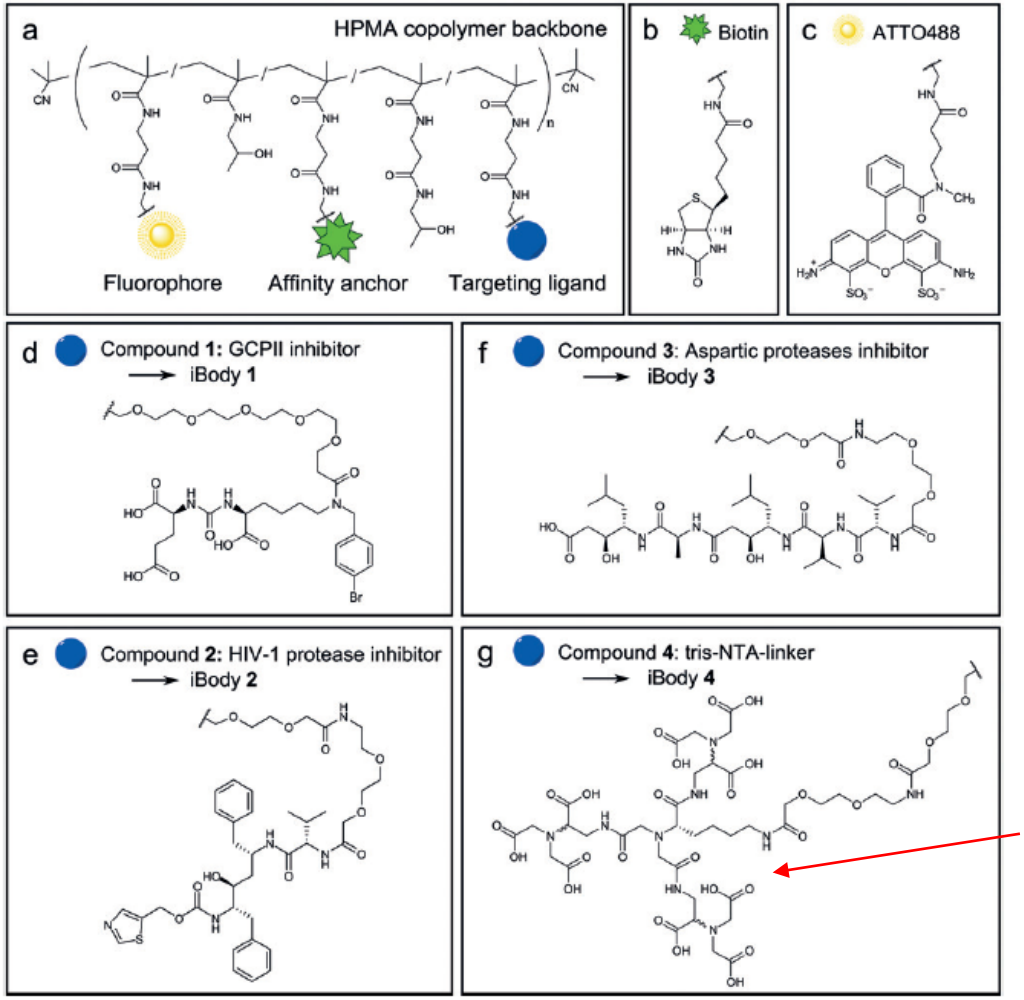


Figure 1. Structures of the iBodies and their functional modules. a) HPMA copolymer decorated with functional molecules. b) The affinity anchor biotin. c) ATTO488 fluorophore. d) GCPII inhibitor. e) HIV-1 protease inhibitor. f) A class-specific inhibitor of aspartic proteases. g) A nitrilotriacetic acid (NTA)-based ligand for binding the His-tag.

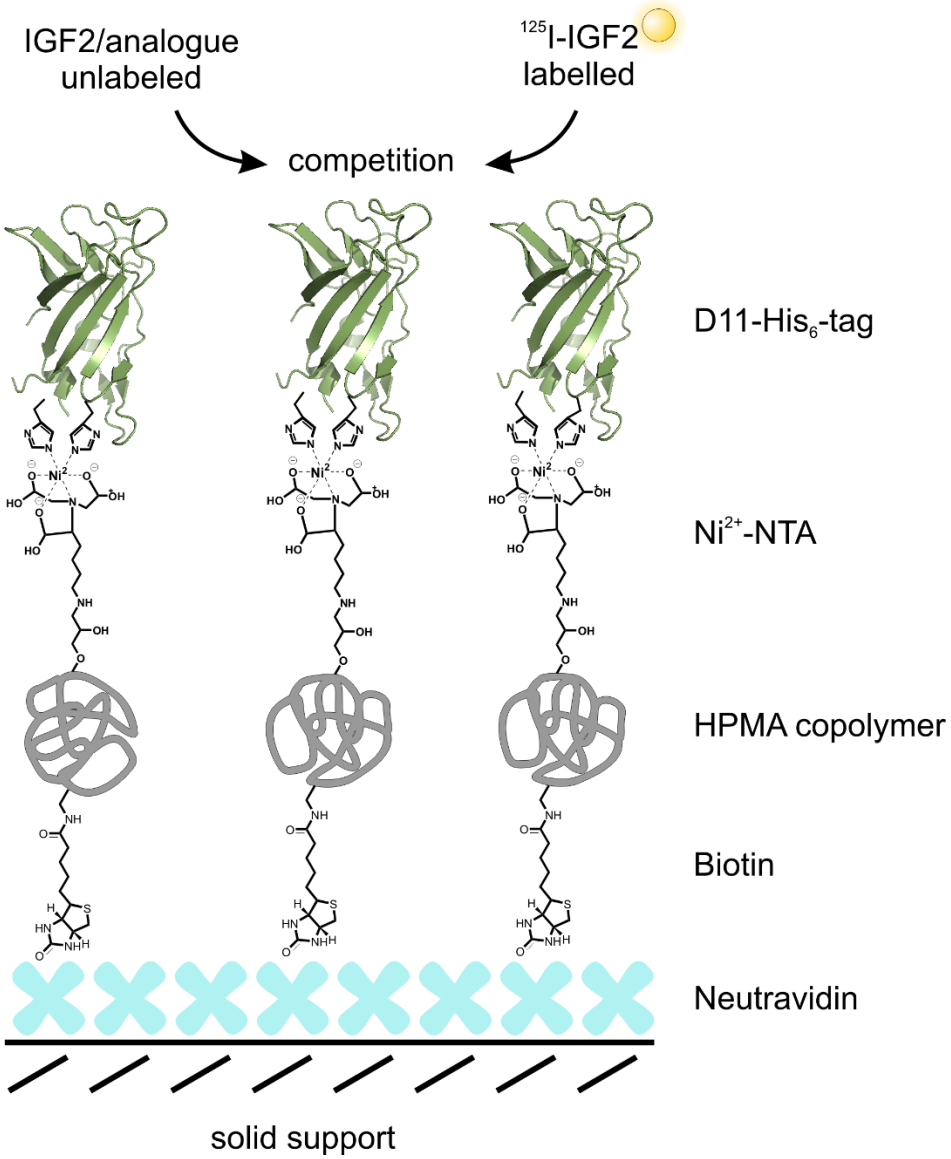
iBodies: Modular Synthetic Antibody Mimetics Based on Hydrophilic Polymers Decorated with Functional Moieties

Pavel Šácha, Tomáš Knedlík*, Jiří Schimer, Jan Tykvart, Jan Parolek, Václav Navrátil, Petra Dvořáková, František Sedlák, Karel Ulbrich, Jiří Strohalm, Pavel Majer, Vladimír Šubr,* and Jan Konvalinka**

Angew. Chem. Int. Ed. **2016**, *55*, 2356 –2360

iBodies kombinují několik způsobů biokonjugace

Ni^{2+}, obdoba afinitní chromatografie na Ni-koloně, konjugace pomocí kovů, specificky se to váže na proteiny s His-tagem



Potalitsyn et al. A radioligand binding assay for the insulin-like growth factor 2 receptor. PLoS ONE 2020